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THE EFFECT OF CHANGES IN ACID-BASE EQUILIBRIUM ON REFLEX TIME

W. W. WENNER AND A. B. TAYLOR

From the Zoölogical Laboratory, University of Iowa

Received for publication June 20, 1929

In a series of studies Travis (1928, 1929) has found that retardation and facilitation of the higher correlation centers respectively decrease and increase the electromyographically determined patellar tendon reflex time.

Grant (1920) in studying the effect of solutions of Na_2HPO_4 and NaH_2PO_4 upon the excitability produced in the sciatic-gastrocnemius nerve muscle preparation of the frog, reported that changes in H ion concentration within physiological limits do not affect excitability. However, the work of Wenner and Blanchard (1928) on the effect of changes in acid-base equilibrium on the physiological action of strychnine and curare, indicated that a change in H ion concentration well within the physiological range might affect some neural or neuro-muscular factor and thus alter the rate of conduction. In order to determine what effect changes in the reaction of the blood might have upon reflex time the experiments reported here were attempted.

The writers take pleasure in acknowledging their indebtedness to Prof. Lee Edward Travis for the use of his laboratory, and for much helpful advice and criticism.

The apparatus used for recording the patellar reflex time of dogs in this experiment consisted of a three stage resistance coupled amplifier, a Westinghouse portable three element oscillograph, a General Radio vacuum tube oscillator and a signal circuit. The set up of this apparatus has been arranged by Travis and Hunter (1928) and is described in their paper.

Large dogs of various ages were used as experimental animals. They were placed on small wooden racks and tied so that the left hind leg remained free to receive the stimulus and to execute the reflex. The electrodes were placed $\frac{1}{4}$ inch apart on the medial surface of the thigh approximately midway between the origin and insertion of the gracilis

and sartorius muscles. A thin strip of brass, which was insulated from the animal by a rubber dam was placed over the patellar ligament. The stimulus, in the form of a blow of an iron hammer on the brass strip which closed the signal circuit produced the reflex which the photographic film recorded. Ten records were taken for each experimental condition of each animal.

TABLE 1

Effect of changes in acid-base equilibrium on reflex time

DOG NUMBER	WEIGHT	GRAMS OF SUBSTANCE GIVEN	pH	CO_2 CAPACITY	REFLEX TIME
	kgm.			*vol. per cent*	*0.001 second*
1	20		7.35	44.7	0.0094
		5 grams NH_4Cl	7.19		0.0059
		7.5 grams $NaHCO_3$	7.47	70.2	0.0062
2	18		7.36	49.4	0.0085
		5 grams NH_4Cl	7.17	22.2	0.0051
		11 grams $NaHCO_3$	7.74		0.0063
3	14		7.29	42.8	0.0086
		5 grams NH_4Cl	7.23		0.0085
		45 mgm. uranium nitrate	7.14	30.4	0.0042
4	13.5		7.34	44.7	0.0082
		5 grams NH_4Cl	7.08	12.8	0.0035
		12 grams $NaHCO_3$	7.68	88.5	0.0054
5	17.5		7.34	38.1	0.0080
		7.5 grams NH_4Cl	7.18	14.2	0.0012*
		5 grams NH_4Cl	7.22		0.0032
		5 grams $NaHCO_3$	7.52	71.8	0.0063
6	16		7.33	43.8	0.0077
		5 grams NH_4Cl	7.26	28.7	0.0046
		5 grams $NaHCO_3$	7.53	72.9	0.0071

* Dog 5 was in oestrus at the time this record was made.

Changes in the rate and intensity of stimulation have no appreciable effect upon the reflex time (Tuttle, 1929). Therefore no attempt was made to keep such factors constant.

The dogs were bled by cardiac puncture and their normal pH and CO_2 capacities determined. Ten records of the patellar tendon reflex of these normal unanesthetized dogs were made.

Acidosis was produced in these experimental dogs by oral administration of five grams of NH_4Cl. When the pH and CO_2 capacity of the blood decreased sufficiently, the reflex time of these animals was again determined.

Several days later, when the effects of the ammonium chloride had disappeared and the pH of the blood returned to normal, all dogs except dog 3 were injected intravenously with 5 or more grams of $NaHCO_3$. Records of the reflex time were obtained immediately after the injection.

Data of the six experimental dogs are summarized in table 1.

Figure 1 shows records of the patellar reflex times of dog 5 under the normal and experimental conditions. Reading from above downward, *A* is the time line in 0.001 second, *B* is the action current and *C* the signal line. The break in the signal line represents the instant of stimulation of the patellar tendon.

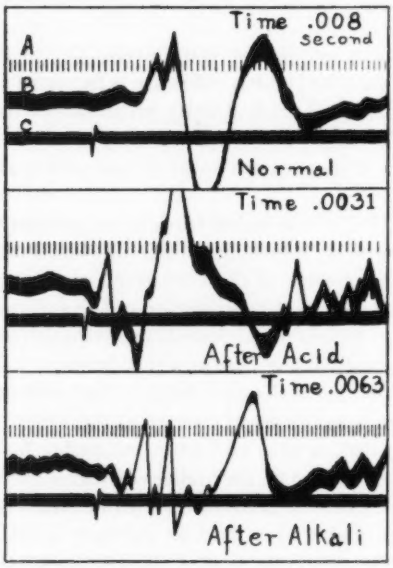

Fig. 1. Records of the patellar tendon reflex of dog 5 under normal and experimental conditions. To facilitate reading the records the diagram on the right is presented.

The patellar reflex time of dogs under normal conditions varied from 0.00775 to 0.0094 second with an average of 0.0082 second. With an increase in H-ion concentration the reflex time was decreased to 0.0045 second. There is no correlation between decrease in reflex time and fall in pH and CO_2 capacity.

An increase in pH and CO_2 capacity following intravenous injections of $NaHCO_3$ likewise reduced the reflex time. In this case, however, the decrease in reflex time was not as pronounced as with NH_4Cl.

Data from dog 5 show that the rate of conduction of a nerve impulse through a reflex arc under experimental conditions may be 6.6 times as fast as conduction through the same arc under normal conditions. This marked change in the rate of conduction might be brought about by conditions affecting the whole arc or any one part of it such as the nerve fiber, the synapse or the motor end plate. Erlanger and Gasser (1924), working on the rate of conduction of the nerve impulse in the sciatic nerve of the bull frog, found that the nerve impulse travels at different rates in different fibers of the same nerve.

Zotterman (1920) reported that the motor end plates conduct with a decrement while the nerve fibers are unaffected by perfusions of acid solutions.

The work of Athanasiu (1925) and Travis, Tuttle and Hunter (1927) indicates that the patellar tendon reflex arc is far more complex than commonly thought and that it undoubtedly involves the higher centers in the cerebral cortex. Though their work was carried out on human subjects it is probable that the higher centers are involved in the patellar tendon reflex arc in dogs.

Burridge (1917, 1928) concluded from his experiments on the effect of perfusing the heart with solutions of different concentrations of calcium and H ions that the passage of the excitatory wave across the synapse is brought about by the relative calcification of the synapse. An increase in H-ion concentration within physiological range would prevent calcium from combining with the colloid particles and thus produce inhibition.

From our results we are inclined to believe that with an increase in H ion concentration there is a decrement created at the synapse or the motor end plates or perhaps both neural and neuro-muscular factors are involved. This would probably block out the higher cortical centers and thus shorten the reflex arc and in this way account for the decreased reflex time.

It would seem probable then that an increase in OH ions produces an effect opposite to that of H ions, that is, the decrement at the nerve endings would be decreased. If such is the case then the passage of the nerve impulse would be facilitated and the reflex time decreased. In this condition the reflex arc itself is not shortened.

SUMMARY

The patellar tendon reflex time of normal unanesthetized dogs was determined and compared with the reflex time of the same dogs after administrations of ammonium chloride and sodium bicarbonate.

With an increase of H ions the reflex time is decreased to a greater extent than when the concentration of OH ions is increased.

A probable explanation is offered in the text.

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CONCERNING THE MOTOR MECHANISM OF THE SALIVARY GLANDS

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From the Department of Physiology, McGill University, Montreal, Canada

Received for publication July 29, 1929

Experiments with the stimulation of the cervical sympathetic nerve (1) (2) (3), and with administration of histamine (4) showed that in the submaxillary gland of a dog and a cat some contractile phenomena are taking place.

What kind of contractile elements are stimulated by the sympathetic nerve and by histamine?

The most natural supposition was that the salivary ducts and maybe the gland itself possess smooth muscles which contract under the influence of the sympathetic nerve and histamine. The physiological analysis of the action of the sympathetic nerve (3) and histamine (5) showed that it is very doubtful that their action is due to the contraction of the muscular fibres.

Further evidence that the contractility of the salivary glands was not due to the presence of smooth muscles within the gland structure was afforded by the histological investigation by Dr. D. J. Bowie, undertaken in our laboratory in connection with this work. He studied the salivary glands in several dogs, cats and rabbits in series of sections stained by the special methods for differentiating smooth muscles and, excepting those belonging to the blood vessels, was unable to find any muscle fibres in the walls of the ducts or anywhere else in the gland of these animals. Among the methods of staining employed were those devised by Van Gieson, Mallory, Pasini, Bebris and Retterer. The same opinion is shared by Prof. K. W. Zimmermann of University of Bern, Switzerland (personal communication).

Thus neither the sympathetic nerve nor histamine can produce their motor effect through the muscular tissue.

The large blood vessels of the salivary glands follow along the salivary ducts and surround them closely (6). It may be supposed that the constriction of arteries under the influence of the sympathetic nerve by means of traction, pulling, etc., could press out the contents of the ducts. This supposition was made highly improbable by the experiments of Sinel-

nikoff (7) performed in our laboratory long ago. Sinelnikoff cut aseptically the vago-sympathetic nerve in the neck in a dog before the experiment, and waited for three days until the vaso-constrictor fibers would degenerate. The stimulation of the sympathetic nerve under these conditions did not produce any constriction of the blood vessels. The augmented sympathetic secretion obtained after stimulation of chorda tympani persisted, however, until the sixth day when all fibres in the nerve degenerated. The same conclusion may be drawn from the experiments in which pituitrin was injected. This substance produced a long continued spasm of the glandular blood vessels and did not press out the content of the gland (5).

The question may arise whether the pressing out of saliva after the injection of histamine could be due to the sudden filling of the blood

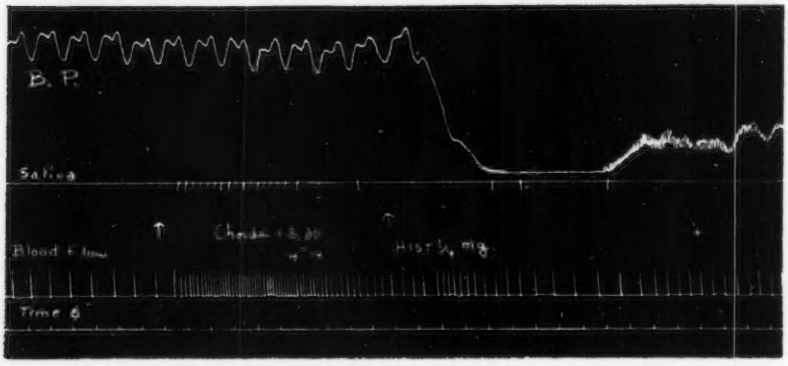

Fig. 1. Cat. Dial. Maevski's method of measuring blood flow through the gland was employed. Capsule of the gland cut open.

vessels of the gland. Such a supposition is legitimate since after stimulation of chorda tympani the passages of the gland are filled with saliva, and histamine further dilates the blood vessels of the gland. Besides this the gland has a capsule which limits the distention.

Special experiments, however, showed that this additional increase of the blood flow through the glandular blood vessels under these circumstances is insignificant, of short duration (*ca.* 10 sec.), and is quickly replaced by an inhibition of the blood flow coinciding with the maximal fall of the blood pressure. This inhibition is in its turn replaced by an increased flow of the blood. The secretion of the saliva usually coincides with the period of the inhibition of the blood flow. The results were the same on a cat and on a dog, not only when the glandular capsule was intact, but also after its removal. One of these experiments is given in figure 1.

Analogous results were obtained also from the plethysmogram of the submaxillary gland of a dog. Like Bunch (8) we have seen the diminution of the volume of the gland following stimulation of either of the secretory nerves. It should be noted that the stimulation of the parasympathetic and sympathetic nerves produced a similar shrinkage of the gland. Injection of histamine into the blood after stimulation of the chorda tympani gave a very short and insignificant increase of the volume of the gland, followed by a marked and long continued diminution. This small increase of the volume of the gland could be due to the initial short dilata-

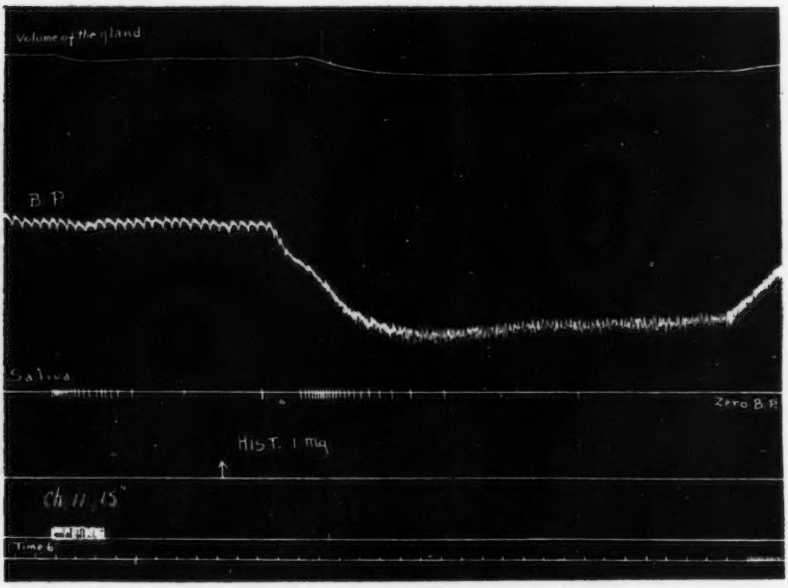

Fig. 2. Dog. Dial. Plethysmogram of the right submaxillary gland

tion of the glandular blood vessels under histamine before the secretion started. One of the experiments with the plethysmographic record of the volume of the gland is present in figure 2.

When histamine was injected without previous stimulation of chorda tympani it produced a very scanty secretion. The volume of the gland in this case changed very little (fig. 3).

Bleeding of the animal (the blood pressure fell from 88 mm. to 47 mm.) did not affect the volume of the gland.

All these experiments speak decidedly against the vascular origin of the contractile mechanism in the submaxillary gland. Since the muscular

tissue can not be responsible for this phenomenon the cause of the shrinkage of the gland must be sought in other mechanisms—histological or physico-chemical. Two histological structures might be supposed to be responsible for the contraction of the salivary glands—the myo-epithelial cells and special structural elements of the secretory cells themselves.

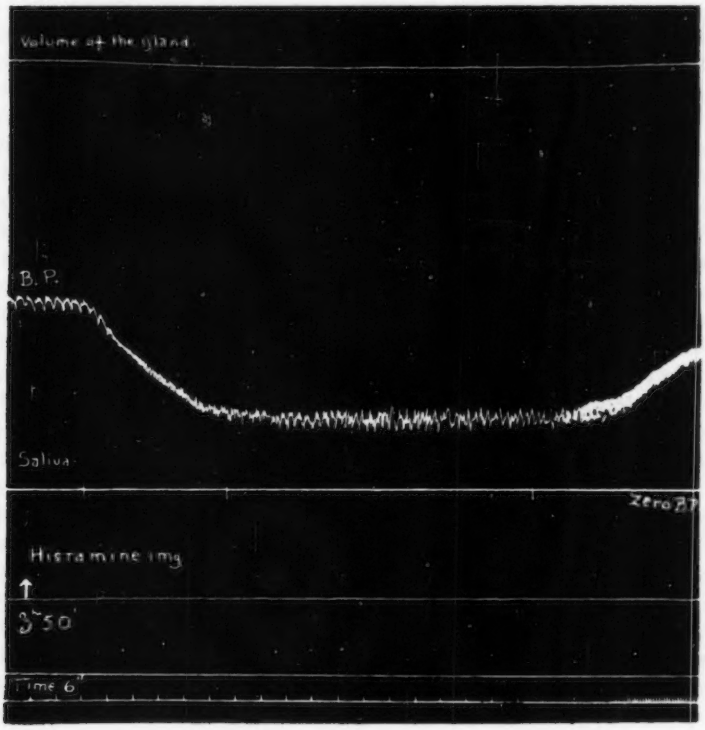

Fig. 3. Same dog as in figure 2. Before the injection of histamine there was a slight spontaneous secretion of saliva.

The myo-epithelial cells are special cells lying between the basal membrane and the base of the secretory cell. They have many processes, which surround the secretory cell. For this reason they have been given the name of "Basket Cells" (Korbzellen). These myo-epithelial cells are not mesodermal smooth muscle cells, but develop from the epithelium. Long ago the property of contractility was ascribed to them. A greater number of myo-epithelial fibrillae surrounds the mucous cells than the serous cells

in mixed salivary glands of man.[1] (The literature concerning the myo-epithelial cells is given by Zimmermann (9) and also by Metzner (10).)

Zimmermann (11) believes that besides the myo-epithelial cells there is another contractile mechanism located in the secretory cells themselves. According to him in the middle of the part of the cell filled with zymogen granules there is located a special nucleus—Diplosoma. This structure according to Zimmermann possesses contractility and presses out the contents of the cell. He calls diplosoma a "Kinocentre," discriminating it from the nucleus which is the "Chemocentre" of the cell. Among other secretory cells he found a diplosoma in the serous and mucous cells of the salivary glands.

Are these histological structures, which may possess a contractility, under the control of the nervous system? A subminimal stimulation of the sympathetic nerve, which does not activate any secretion of saliva, may press out the content of the gland or produce a shrinking of the organ. It would be quite legitimate to admit that the cervical sympathetic nerve besides secretory fibres supplies the salivary glands with motor fibres. What kind of contractile elements it innervates is not known. On the other hand it is very difficult to picture a physico-chemical process which would produce these results.

Far more difficult to explain is the diminution of the volume of the gland under the influence of the parasympathetic nerve. Bunch (8) looked upon the action of chorda tympani on the secretory epithelium as on a true secretory effect. According to him "the effect of secretory nerves is simply and solely upon the secretory cells." And what is more important the first effect of the stimulation is on these cells. Later on they replenish their fluid losses from the lymph spaces and blood vessels. Thus before the blood vessels begin to dilate and supply the secretory elements with fluid, the cells have already discharged a part of their contents into the ducts. Bunch showed that after complete cessation of the blood flow through the gland (ligation of both carotids and both subclavians) the volume of the gland diminishes on repeated stimulation of chorda tympani. When the blood flow through the submaxillary gland is somewhat diminished (occlusion of the carotid artery) there is a very little effect upon the amount of saliva obtained, and no change in the length of the latent period of the secretion can be noticed on the curves (12). Under normal conditions the latent period of the secretion activated by stimulation of chorda tympani is usually a little shorter than that for the increase of the blood flow, as can be seen in many Gesell's curves (13). See figure 1 of this paper too. Barcroft (14) noticed the same relations using adrenaline. A "pressing

[1] Recently Prof. K. W. Zimmermann informed me that the myo-epithelial cells are present ("gut ausgebildet") in the submaxillary gland of both dogs and cats (iron-hematoxylin).

out" effect could be seen on a pilocarpinized submaxillary gland on stimulation of chorda tympani. Gesell (15) attributed this effect to the contraction of the ducts. Chorda secretion was observed in an animal's head severed from the body (16).

These facts together with occasional observations indicate that the parasympathetic nerve undoubtedly has something to do with the contraction of the gland.

In connection with this problem several important questions arise. Are all the chordal motor phenomena described above of the same nature? Could they not be explained by some special physico-chemical changes occurring in the secretory cells immediately after the beginning of the stimulation of the parasympathetic nerve, which later on would be replaced or complicated by processes of osmosis or dialysis? Does the chorda tympani supply the gland with secretory fibres only or with special motor fibres too? Certain facts seem to be in opposition to this idea. Thus the volumetric curves of secretion under stimulation of the chorda tympani and sympathetic nerves are quite different. In the first case the curve rises gradually and has a S-shaped form, in the second case the curve quickly reaches its peak (3). After atropinisation of the gland stimulation of chorda tympani does not press out the content of the gland. This fact shows that if there are motor fibres in the parasympathetic nerve they are paralysed by atropin.

The solution of all these problems must be left for future investigations. We must confess that even such a well known process as the secretion of saliva under the stimulation of chorda tympani is very far from being understood.

In connection with this problem it is interesting to note that the motor phenomena in the pancreatic gland have a different character and are regulated chiefly by the parasympathetic nervous system (Babkin, 17). Only small myo-epithelial cells were found and those only in the main duct of the pancreatic gland (Zimmermann, 9), but it seems that the main pancreatic duct and its chief divisions possess some smooth muscular fibres (Laguesse, 18).

SUMMARY

Motor phenomena under the influence of the stimulation of nerves and action of histamine could be observed in the submaxillary gland of both dogs and cats. No smooth muscles could be found either in the ducts or elsewhere in the gland. The possibility of contraction of other histological elements is discussed. The cervical sympathetic nerve besides secretory fibres possesses motor fibres for the submaxillary gland. No adequate explanation can be given at the present time for the motor effect produced by the stimulation of the chorda tympani.

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CHEMICAL STUDIES ON THE SPLEEN

III. The Action of Splenic and Other Tissues upon the Removal of Hemoglobin-Methemoglobin Solutions

G. B. RAY and L. A. ISAAC[1]

From The Department of Physiology, Western Reserve University Medical School, Cleveland, Ohio

Received for publication July 29, 1929

The first two papers of this series (Ray and Stimson, 1927; Ray, 1928) established two facts: 1, that the spleen is capable of reducing methemoglobin, and 2, that the removal of the spleen is associated with a loss of function of a part of the hemoglobin of the blood. The accumulation of this nonfunctional form presents further proof of the reducing action of the spleen for Stimson (1928) has found the nonfunctional form may be reduced to hemoglobin by the action of hydrosulfite. On the other hand since Stadie's method (1920) has also been used for its determination, it is probable that it can be converted to methemoglobin.

No suggestions have been offered as to the rôle of the spleen as a reducing agent or as to the manner in which it normally acts to prevent the accumulation of nonfunctional pigment. Since the nonfunctional pigment is apparently the result of an oxidative change it is probable that both phenomena are reflections of the same activity. In this research we have tried to determine this function of the spleen and to compare this activity with that of other tissues. It is obvious that such a study must be made under carefully controlled conditions, and therefore is best done *in vitro*. It is also desirable that this study be substantiated by similar reactions involving only known constituents. We therefore carried out experiments of three types in which we used methemoglobin as the pigment, thus providing an oxidized nonfunctional form of hemoglobin. The first type involves the action of an iron salt on methemoglobin, the second the action of various tissues, and the third the extracts of these tissues. In this manner, starting with a reaction where both constituents are known, and leading to a reaction where one is unknown, a firm foundation is laid for the study of the action of tissues, or their extracts on an unknown form of hemoglobin.

[1] Crile Scholar.

Previous work. Considerable work has been reported upon the action of tissues or their extracts on methemoglobin, the most recent of which are the experiments by Neill (1925) and Sakurai (1924–25). Neill (1925) studied the action of bacteria and tissues on hemoglobin-methemoglobin mixtures and found these capable of producing an oxidation (i.e., hemoglobin→methemoglobin) provided the oxygen tension was high. Under conditions of low oxygen tension reduction took place (methemoglobin →hemoglobin). These experiments are not in agreement with those of Sakurai (1924). He apparently paid no attention to the O_2 tension of his solution, yet in every case found a reduction of methemoglobin. However his experiments are open to criticism for he depended upon 0.1 per cent chloroform to produce and maintain sterility. Such a concentration of chloroform is usually regarded as too low for efficient action. It is well to point out at this time an error that appears in Sakurai's second paper (1925) on the removal of methemoglobin by the perfused lung. His results appear opposite to those of Neill which is due to an erroneous use of the Stadie method for methemoglobin.

Sakurai based his measurements on a comparison of the methemoglobin containing blood with a normal standard. However in his calculations he failed to use the O_2 capacity of his standard blood but used the O_2 capacity of the unknown blood. The result is an enormous error. He finds, to cite one measurement, a 29.4 per cent removal of methemoglobin; correctly calculated his results show an increase of 152 per cent during the perfusion. This calculation shows his results to be those that might be predicted from Neill's experiments.

General technique. Dog's blood was used throughout these experiments. In most cases it was drawn from the jugular under aseptic conditions with a sterile syringe. Oxalate was used to prevent clotting. In some of the experiments the blood was taken from animals used n other experiments. These animals were bled from the heart with a sterile glass cannula. Anesthesia previous to bleeding did not seem to have any effect on our experimental results. The cells were centrifugated in sterile tubes and washed with sterile saline. When more than enough for one experiment was taken they were stored in the cold until used. It will be observed that throughout care was taken to maintain an aseptic technique. The same applies to the other phases of the experiments. All glassware and other materials coming in contact with the blood were well sterilized before use. When laking was necessary it was done by the addition of sterile distilled water.

Methemoglobin was produced by the addition of either potassium ferricyanide, sodium nitrite or amyl nitrite. The latter compound was used most frequently since the end products are either volatile or innocuous. The per cent methemoglobin was determined by Stadie's (1924) method,

and the O_2 capacity by the constant volume Van Slyke-Neill apparatus. The limit of variation allowed between check readings of O_2 was 0.2 volume per cent. The readings of the Stadie method were followed by means of a spectrophotometer. A Keuffel and Esser "Color Analyzer" was used for this purpose and as will be shown in another paper proved to be very dependable. The blood was diluted 1:100 with 0.4 per cent ammonia for spectrophotometric observation and where certain samples were to be compared the dilution was adjusted until the total pigment content of each flask was equal to the others. In this manner the percentage change in pigment in each sample was obvious and the curves could be compared with ease. All readings in this paper are given in terms of the optical density, i.e., the negative logarithm of the light transmitted.

Blood treated as outlined above was pipetted into Erlenmeyer flasks usually 25 cc. and the reagent or saline added. Where the experiments were aerobic the blood was oxygenated and the flask loosely stoppered with cotton. When anaerobic conditions were necessary the oxyhemoglobin was reduced by passing nitrogen through the solution and the reaction was carried out in an atmosphere of this gas. In these experiments the flasks were closed with rubber stoppers. Incubation lasted from 16 to 72 hours and was always at 37°C. The changes occurring in the methemoglobin content were calculated on a basis of the incubated control flask and not on the preliminary reading.

Experiments with iron. The activity of iron salts, particularly the ferricyanides, in the production of methemoglobin has been the basis of considerable investigation (von Reinbold (1913) Meier (1923); Conant and collaborators (1924–25–28)). These investigators have chiefly concerned themselves with the action of ferric compounds on oxyhemoglobin. While Conant (1924–5) points out that the formation of methemoglobin from hemoglobin is a reversible reaction, comparatively little attention has been paid to this phase. In our experiments we are not so much concerned with the equilibrium which will be reached between equimolar concentrations of iron and hemoglobin as we are with the reversibility of the reaction. It is much more pertinent to our experiments if we can show that under one set of conditions we have an oxidation and if we change those conditions, particularly the O_2 tension, we can reverse the reaction. If this can be done we will have demonstrated the capability of an iron salt to behave in the same manner as Neill found biological material to behave. In order to demonstrate this point we have tested the effect of a ferrous salt on a mixture of hemoglobin and methemoglobin and observed the result of a change in O_2 tension.

The washed cells for these experiments were treated with sufficient amyl nitrite to produce about 50 per cent methemoglobin. In order that the reaction might be fully complete the mixture was stored in a cool

place for 12 hours. After a preliminary determination of methemoglobin content the blood (50 cc.) was placed in a tall cylinder. A current of nitrogen was forced through the blood until nearly all of the oxygen was removed as shown by a Van Slyke determination. At this time ferrous chloride made by the addition of an excess of pure iron wire to a known amount of hydrochloric acid was added to the blood in sufficient concentration to completely reduce the methemoglobin, provided one equivalent of ferrous iron completely reduced one equivalent methemoglobin. The reaction was followed in two ways; by means of the spectrophotometer and by means of the changes in oxygen capacity. The reaction was continued for ½ hour at which time it was adjudged complete and a large sample was pipetted into a tonometer for O_2 capacity measurements.

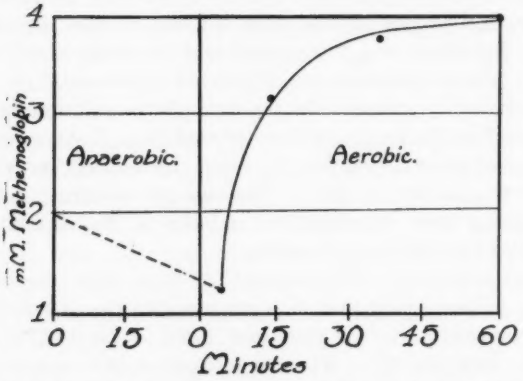

Fig. 1. Changes in methemoglobin concentration under aerobic and anaerobic conditions where the blood pigments are allowed to react with ferrous chloride.

Figure 1 presents, in a graphical form, the results of a typical experiment. No readings are given for the 30 minute anaerobic period and the direction of the reaction is indicated by a dotted line. The direction of this line is determined by the position of the first reading of the aerobic period which was made four minutes after oxygenation of the blood. The position of this point is not accurate since the succeeding points on the curve of aerobic activity indicate a continuous formation of methemoglobin. However, since the first aerobic reading is in that portion of the curve where the reaction is most rapid it is probable that the actual concentration of methemoglobin is much lower than indicated. It must be emphasized at this time that we consider our results as directional only. We have not attempted to establish the equilibrium between equivalent concentrations, nor have we attempted to show the quantitative relationships of one

substance to another. We feel that it is only pertinent to our thesis that we demonstrate the removal or formation of methemoglobin. Since the concentration of methemoglobin is much lower at the first aerobic reading than at the preliminary reading, there can be no doubt that it is removed by the action of the ferrous salt.

The reverse is true in the aerobic phase. Each point on the curve represents a single O_2 capacity determination and each reading was preceded by an oxygenation. The reaction, i.e., the formation of methemoglobin, continues until practically all of the hemoglobin is converted to methemoglobin.

There are certain significant features about these results: 1. In a mixture of hemoglobin and methemoglobin, methemoglobin is removed by a ferrous salt where the oxygen tension of the solution is low. It is probable that during this period the ferrous salt is converted to a ferric form just as the ferric hemoglobin (methemoglobin) is converted to ferrous (hemoglobin). 2. Upon the admission of O_2 the reaction is reversed. The ferric salt, resulting from the reduction of methemoglobin, plus the admitted O_2 acts as an oxidizing agent. This results in an oxidation of hemoglobin to methemoglobin. A point of additional interest, in this connection, is the excess formation of methemoglobin. It will be recalled that only sufficient ferrous chloride was added, to convert the methemoglobin (2 mM.) present to hemoglobin if 1 equivalent of iron reacted with 1 equivalent of pigment. The change in oxygen capacity of the solution shows practically all of the hemoglobin to be oxidized. This is far more than one would predict from the amount of iron added. It is, therefore, probable that the iron is acting as a catalyst. This is readily explained by the fact that many iron salts are unstable in the ferrous form and in the presence of O_2 pass into the ferric state. These may be complex salts of high oxidizing potential. In the presence of a reducing substance these complex salts would go to the ferrous form once more. This process might go on indefinitely; the end of the reaction being determined by the complete oxidation of the reducing compound. The action of the ferrous chloride is similar in its behavior to that of sodium hydrosulfite in the Van Slyke method for methemoglobin wherein the hemoglobin formed must be fixed or reoxidation occurs.

Experiments with tissues. Having established the action of iron under our experimental conditions we directed our attention toward biological material. The simplest and most direct manner of studying the action of a tissue is to introduce the tissue itself into the reaction flask. This, however, is attended by certain difficulties. There is the omnipresent danger of bacterial contamination which increases with the amount of manipulation. Secondly, there is the question of the proper contact between the tissue and solution. The reaction must take place at the surface of the

tissue and with a single piece the area is small. Sakurai attempted this method and ground the tissue with sand. The criticism to his results has already been advanced. In our own experiments we employed various methods, none of which were attended by a high degree of success.

The first experiments were the most complicated since we hoped to follow the course of the reaction and plot a time curve. The apparatus consisted of a percolator system where the blood dripped over the tissue. It was then pumped through an oxygenating device into a reservoir from which it dripped on the tissue a second time. When we found methemoglobin formed in the course of the experiment the attendant danger of contamination forced us to discard the results.

The technique eventually adopted was the simple addition of a small piece (about 10 grams) of sterile tissue to the solution of blood pigments. It is true that this was not conducive to good contact between the tissue and blood but the freedom from contamination and the advantage of being able to control the O_2 tension outweighed this disadvantage.

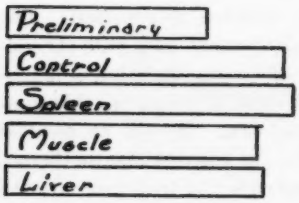

Fig. 2. The effect of various tissues allowed to react on a solution containing hemoglobin and methemoglobin.

The results of an experiment of this nature are presented in figure 2. The blood was treated in the same manner as that used in the iron experiments. In those flasks in which the reaction was to be aerobic the cotton plug used for sterilization was left intact and the flasks shaken from time to time to insure oxygenation. In order to produce an aerobic condition we found tightly sealing the flask was sufficient, since the metabolism of the tissue removed the O_2. Following the mixture of the components and a preliminary determination of hemoglobin content the flasks were incubated at 37°C. or 16 to 60 hours. The results presented in figure 2 are characteristic of all our experiments and are obviously not conclusive for the control flask, where only saline was added, shows as much change as any of the others. In these obsevations we are in agreement with Sakurai who found a spontaneous removal of methemoglobin. Neill apparently did not make a control reading after incubation; it is possible that had he done so he would have come to the same conclusion.

Experiments with sterile extracts (aerobic). The lack of positive results with tissues led to the adoption of another technique wherein better contact would be established betwen the active portion of the tissue and the record. In order to do this we used either tissue juice or extract. The results are calculated from the second control, i.e., the reading after incubation. The following types of extracts were tested:

Extract no. 1. Five hundred grams of muscle from a cat, anesthetized with ether and bled to death was chopped in a food chopper then titurated with sand and placed in a canvas bag. The ground muscle mass was extracted in a Buchner press at a pressure of 4300 lbs. per square inch and the resulting liquid was rendered sterile by filtration through a Berkefeld N filter. The table shows that the extract alone had only a slight effect on the hemoglobin. Splenic tissue (Ib) which was studied at the same time also had no effect. In that flask in which both tissue and extract were combined (Ic) we find 2.92 millimols of hemoglobin converted to methemoglobin. Apparently this synergic action is the result of one substance supplying some factor lacking in the other. It is possible that filtration through the Berkefeld filter had an influence on the muscle extract.

Extract no. 2. The acetone extract of muscle was made by extracting the tissue for 6 hours in the Soxhlet apparatus with acetone. The acetone was evaporated and the gummy mass taken up in saline and shaken to emulsification. The table shows the extract so made to have only a feeble action.

TABLE 1

EXTRACT	ORIGINAL HEMOGLOBIN	FINAL HEMOGLOBIN	METHEMOGLOBIN
	mM	*mM*	*mM*
Ia. Muscle juice	7.34	6.96	0.38
Ib. Spleen tissue	7.34	7.10	0.24
Ic. Ia and Ib	7.34	4.42	2.92
II. Acetone extract	8.53	8.09	0.44
III. Acetone insoluble ether solution	6.10	6.03	0.07
IV. Acetone insoluble water solution	6.10	6.32	−0.22
V. Alcohol solution	6.10	3.59	2.51
VI. Acetone insoluble water solution	3.49	3.36	0.13
VII. As V buffered	3.49	1.95	1.54
VIII. Alcohol spleen	3.49	0.84	2.65
IX. VII and VIII	3.49	0.82	2.67

Extract no. 3. One part of the acetone extracted tissue was replaced in the cup of the Soxhlet apparatus and re-extracted with ether. The extracted material was treated in the same manner as the acetone extract. It will be seen that the extract made in this way is not active.

Extract no. 4. The portion of the acetone extracted material not used for the ether soluble fraction was put in 10 times its volume of distilled water and boiled for 2 hours. The tissue was filtered off and the filtrate reduced to 30 cc. on the stem bath. This extract gave no results.

Extract no. 5. Muscle tissue, after mincing, was boiled with 5 times its volume of freshly distilled absolute alcohol under a reflex condenser for three hours. It was then filtered and the alcohol driven off from the filtrate on the steam bath. The residue was taken up in 50 cc. of saline. The resultant solution was a thick russet brown suspension; 5 cc. of this suspension in 25 cc. of blood converted 59 per cent of the hemoglobin to methemoglobin.

Extract no 6. This extract is the water soluble, alcohol insoluble fraction of muscle. The residue from the filtration of the extracted muscle mass in the preparation of extract 5 was boiled with 5 times its volume of water. The resulting infusion was filtered through flannel. The filtrate was evaporated on the steam bath and the

residue taken up in saline which had a mixture of phosphates added to bring the pH to about 7.5. This was shaken to a permanent suspension. The table shows the extract to be inactive.

Extract no. 7. This extract was prepared in the same manner as extract 5 with the exception that the saline used to take up the extract contained phosphate buffers giving a pH of 7.5. This extract produces a reaction, but this reaction is only slightly greater than that observed in the case of the unbuffered (40 per cent conversion against 44).

Extract no. 8. An extract of splenic tissue (sheep's spleen) made in the same manner as the preceding. This made a potent extract as will be seen from the table. Five cubic centimeters in 25 cc. of blood converted 64 per cent of the hemoglobin to methemoglobin.

Extract no. 9. Since in experiment Ic of this series there seemed to be a synergic effect as a result of combining spleen tissue and muscle extract it is interesting to test the combination of the two extracts. This has been carried out in this experiment and we find no increase in oxidation over that observed for splenic extract alone.

These results show clearly that the alcoholic extracts are most effective in action since no difference could be observed between the buffered and unbuffered extracts, due without doubt to the extraction of buffer salts from the tissues; we used a saline suspension of the alcoholic extract for all other experiments reported in this paper. These experiments with extracts were repeated several times with consistent results and in the later experiments a comparison was made with liver extract made in the same way.

Figure 3 shows the relative activity of the three extracts as well as the spontaneous activity of the blood itself in relation to methemoglobin. No definite figures have been given for the extent of the reaction for the general picture can only be considered qualitative. In order to compare experiments it would be necessary to devise some means of standardization of the extracts which would involve considerable labor irrelevant to the main thesis. As in the case of our experiments with iron the results are only directional. It is significant that never in the presence of O_2 did we find a removal of methemoglobin which is contrary to the results of Sakurai but is in an agreement with the action of other material as shown by Neill.

In confirmation of the above results, obtained by the Van Slyke and Stadie methods, the results formed by spectrophotometric examination are given in figure 4. The tube length was 10 mm. and check readings by a second observer gave very close agreements. The results are plotted on semilog paper in terms of the reciprocal of the light transmission (optical density).

Curve A is the absorption curve of blood from the control flask where 5 cc. of saline had been added to 25 cc. of blood previously treated with amyl nitrite. The incubation period for this experiment was 40 hours.

The methemoglobin may be estimated from the ratio of the extinction coefficient at 540 to that at 560 $\frac{-\log T540}{-\log T560}$. From Huefner's table (1900) one may determine the per cent methemoglobin. From this curve we find it to be 36 per cent.

Curve B is that of the same hemoglobin-methemoglobin mixture allowed to react with muscle extract. It will be noted that the absoption at 540 and 575 millimicra is decreased as compared with the control curve. This

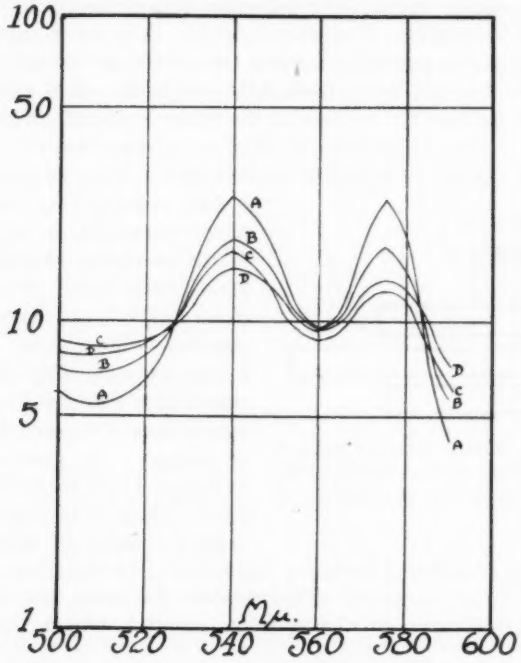

Fig. 3. Absorption curves of hemoglobin-methemoglobin solutions allowed to react with various extracts.

is characteristic of an increased amount of methemoglobin. The ratio $\frac{E_{540}}{E_{560}}$ has a value of 1.38 in this curve which is equivalent to 50.4 per cent methemoglobin.

Curve C is that found for the reaction with liver extract. Its action is somewhat greater than that of muscle and the ratio equals 1.32 which corresponds to 64.2 per cent methemoglobin. Splenic extract (curve D) is of relatively the same potency as liver. The ratio equals 1.33 which according to Huefner is equivalent to 63.13 per cent methemoglobin.

The experiments given above show conclusively that, in the presence of oxygen, the alcoholic extracts of tissues oxidize hemoglobin to methemoglobin. It is of importance in connection with our thesis to note that the spleen produces an extract of an activity comparable with those of other tissues. This is contrary to Sakurai's results. He found splenic tissue to be relatively weak as a reducing agent.

Anaerobic experiments with extracts. The results of the foregoing section led naturally to the question of the influence of oxygen tension on the direction, i.e., either oxidation or reduction of the reaction between tissue extracts and hemoglobin or methemoglobin. It is much more pertinent to demonstrate the capability of the tissues to remove methemoglobin than to show their ability to form methemoglobin. It is essential to an understanding of how the absence of the spleen can cause an accumulation of an oxidized form of hemoglobin that we demonstrate the tissue or its extract to be capable of removing methemoglobin from solution.

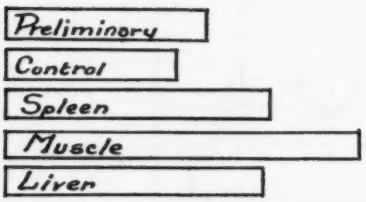

Fig. 4. The relative effect of various tissue extracts acting on methemoglobin-hemoglobin solutions in the presence of oxygen.

The technique of these experiments was similar in nature to that of the preceding section with such additions as found necessary to remove and exclude oxygen from the reaction. The laked cells after treatment with amyl nitrite were placed in a graduated cylinder and washed free of oxygen with a current of nitrogen. In some experiments hydrogen was substituted for nitrogen. When the hemoglobin was judged sufficiently reduced, 25 cc. portions were transferred to flasks which had previously been filled with nitrogen. During the period of the reaction the flasks were either closed with a rubber stopper or the solution covered with a thick layer of paraffine oil.

Table 2 shows the results of certain experiments of this type. The blood used in these experiments of table 2-A was concentrated to half its original volume by centrifugation and treated with nitrous oxide and allowed to stand 20 hours at 5°C. The original concentration of hemoglobin was 10.40 mM. and the final concentration 7.72 mM. Hence the concentration of methemoglobin was 2.68 mM. Each flask contained 25 cc. of the blood. Five cubic centimeters of extract were added to each flask and the same amount of saline to the control. The reaction time was 57 hours.

Table 2-B represents an incubation of 16 hours. The extracts used in this experiment were the same extracts as those reported in table 1. The

methemoglobin was produced by the addition of potassium ferricyanide. The changes in methemoglobin content are opposite in direction to those observed in the presence of oxygen. The exception to this action is found in the case of liver extract which shows an increase of 0.98 mM. The control also shows an increase of 0.65 mM.

It is characteristic of all of our experiments that, while liver extract formed methemoglobin in the presence of oxygen, the absence of oxygen failed to change the direction of the reaction. It appears that liver extract contains a potent oxidizing substance capable of acting regardless of the oxygen tension.

Both splenic and muscle extracts produced a decrease in the amount of methemoglobin in solution. In the case of the extract of spleen this

TABLE 2A

FLASK	ORIGINAL METHEMOGLOBIN	FINAL METHEMOGLOBIN	PER CENT CHANGE*
Control	2.68	3.33	—
Spleen	2.68	2.18	−29
Liver	2.68	3.66	+30
Muscle	2.68	1.90	−44

* As compared with control.

TABLE 2B

EXTRACT	ORIGINAL METHEMOGLOBIN	FINAL METHEMOGLOBIN	PER CENT CHANGE
Control	5.30	7.62	—
Spleen	5.30	4.63	−39
Muscle	5.30	4.63	−39

amounts to 0.50 mM. when referred to the original concentration and 1.15 mM. when compared with the incubated control. This particular muscle extract was more active than the splenic, which was not always the case. We find in the experiment cited, that this muscle extract was capable of producing a reduction of 1.43 mM. or 44 per cent of the methemoglobin present in the solution. It has already been mentioned that these experiments are to be considered directional in nature and the quantities measured are only indicative.

The significant point in these experiments is the marked reduction of methemoglobin in anaerobiosis. This is shown clearly in figure 5 where the relative activity of the extracts is shown. Comparing this with figure 2 we find the results, with the exception of liver, to be directly opposed.

The results of the extract activity are comparable to the action of benzaldehyde in the experiments of Baeyer and Villiger. These investigators found benzaldehyde converted to benzoyl hydrogen peroxide in the presence of oxygen. This compound is capable of oxidizing other compounds, even benzaldehyde. The action is not reversible for the end product is benzoic acid. A similar type of activity has been observed by one of us (Ray, 1927) in the oxidation of sodium lactate by hydrogen peroxide. The active material in this case is supposed to be acetyl peroxide, which not only attacks the lactate but also the hemoglobin. It is significant, in the last cited experiments, that, when oxygen was excluded from the reaction flasks, no oxidation of hemoglobin took place.

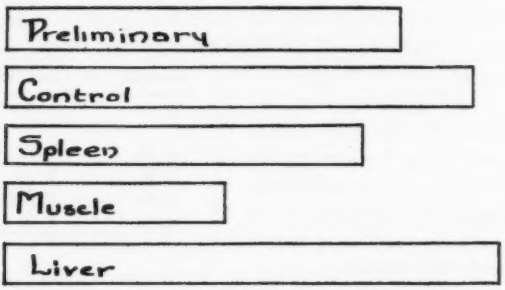

Fig. 5. The relative effect of various extracts on hemoglobin-methemoglobin solutions in the absence of oxygen.

Each of the above instances shows the importance of oxygen for certain types of oxidative reactions. In the case of acetaldehyde the parallelism is quite close for the action of the peroxide on hemoglobin results in methemoglobin.

SUMMARY

1. Ferrous chloride reduces methemoglobin to hemoglobin in the absence of oxygen. This action is reversed in the presence of oxygen.

2. The alcoholic extracts of spleen and muscle behave in the same manner as ferrous chloride.

3. Liver extract produces an oxidation of hemoglobin regardless of the oxygen tension.

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A STUDY OF RESPIRATORY AND CIRCULATORY RESPONSES TO A VOLUNTARY GRADUAL FORCING OF RESPIRATION

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At the time that we were studying the effects on man of a slowly developed anoxemia and of a gradual increase of carbon dioxide in the inhaled air, it seemed also desirable to be able to compare these two sets of reactions with physiologic responses of a gradually produced acapnia, which is a lowering of the partial pressure of carbon dioxide in the lungs and hence in the blood. For this purpose we had men and women breathe the air of a Larsen-Henderson rebreathing apparatus in order that the minute-volume of breathing might be exactly guided. With this apparatus the breathing was recorded on a kymograph, which made it possible for the operator to direct the subject from minute to minute as to the amount of increase that should be made in his breathing. The results of our study have recently come to be of interest in view of an investigation by Vincent and Thompson (1928) on blood pressure in hyperpnea.

In 1927 Collier, Densham, and Wells investigated the effect of overventilation of the lungs on the blood pressure and concluded that the systolic pressure remained almost constant in man, during forced breathing, by means of a compensatory cutaneous vasoconstriction. Vincent and Thompson, on the other hand, report that a large majority of men show a fall of blood pressure during excessive ventilation of the lungs, which they attribute to a mechanical interference with the return of blood to the heart, and also, in a small degree, to a lowered carbon dioxide tension in the blood.

Most of the earlier investigations of the effects of forced breathing were made upon anesthetized animals. The most extended of these were by Henderson (1908–1910), who published a series of papers that deals with the circulatory failure and general loss of vitality produced in the dog by excessive artificial ventilation. The phenomenon of the fall in arterial blood pressure has been studied with anesthetized cats by Dale and Evans (1922).

Experiments on man have also been reported from Henderson's laboratory (Henderson, Prince, and Haggard, 1918, and Henderson and Harvey, 1918). These show that voluntary forced breathing has a depressant ac-

tion on the circulation but that the effect is neither upon the heart nor upon the vasomotor mechanism, for the heart is accelerated and the arterial pressure is not usually lowered. It is concluded that the circulation is rendered slower by a decrease in the venous return of the heart.

EXPERIMENTAL PROCEDURE AND RESULTS. The studies here considered were undertaken particularly as a contrast to a group of experiments where the subjects breathed an excess of carbon dioxide. In these experiments with an excess of carbon dioxide, the subject rebreathed the air of a Larsen-Henderson rebreathing apparatus in which the expired carbon dioxide was gradually allowed to accumulate to the point where breathing became so labored and difficult as to be distressing. Low oxygen effects were eliminated by constantly introducing into the apparatus enough pure oxygen to maintain the normal oxygen content.

In the acapnia experiments an effort was made to exactly duplicate the conditions of the carbon dioxide runs, except that the carbon dioxide was absorbed by a sodium hydroxide cartridge and the impetus for deeper breathing was the voluntary effort of the subject. The operator of the rebreathing machine had before him a carbon dioxide run for the same subject and guided the subject in his voluntary breathing to make an increase, duplicating minute by minute in amount and time the carbon dioxide run. At the beginning of each experiment the subject breathed naturally for three minutes to establish a normal and then gradually augmented his breathing. During the last two minutes of the run, the subject was instructed to breathe as much as he possibly could. The termination of the run was determined more by the subject's own feelings than by anything else, the run being terminated when signs of tetany were observed. The average length of run was 22.2 minutes.

A total of 61 experiments was made on fourteen different subjects. Determinations were made of the following respiratory and circulatory reactions: alveolar air; minute-volume of breathing; arterial, venous, and capillary blood pressures; hand volume; and the blood flow through the hand.

Subjective symptoms. The subject at first experienced no difficulty in increasing the breathing; but after a few minutes there usually came a stage when there was a tendency to flag, prodding by the operator became necessary and drowsiness was frequently noted, sometimes in the extreme. Later the deeper breathing seemed easier, but at about this time there was a tendency to notice the symptoms due to excessive ventilation. This would be first noted as a tingling sensation in the fingers or possibly the toes, and sometimes in the face. Occasionally there was a twitching of the muscles of the face. Later there was a cramping of the fingers. When these symptoms became at all marked the subject was always removed, since the symptoms were apt to become worse immediately

after removal. In a few cases there was a cramping of the chest which impeded breathing at the end of the run, the subject being under the impression that breathing was increasing properly, while the spirometer registered up and down excursions of less than an inch and the chest of the subject showed rapid and violent but ineffectual inspiratory attempts. The color of the subject did not appear to change, nor was there any tendency to lose consciousness.

The most striking symptoms were usually experienced afterward, when the hands and feet of the subject often became tense and rigid and in one

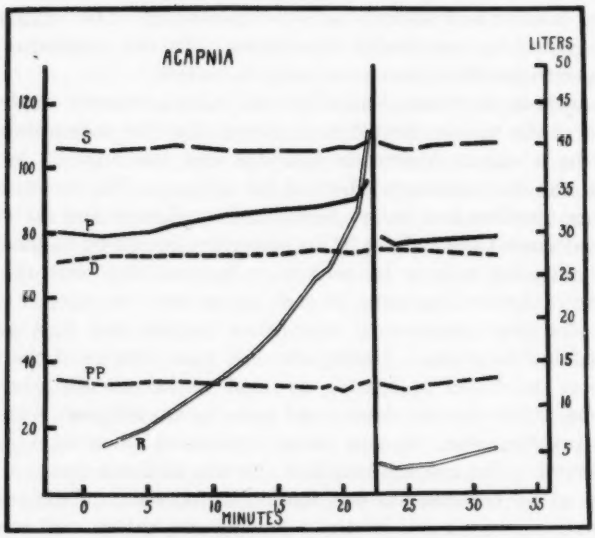

Fig. 1. Curves of the arithmetical means of the circulatory response of 60 cases to a gradual increase in breathing. *P*, pulse frequency; *S*, systolic pressure; *D*, diastolic pressure; *PP*, pulse pressure; and *R*, respiratory volume per minute.

or two instances the chest muscles became so spastic that breathing was for a time impossible. In one instance the shoulders were numb and cramped for fifteen minutes after the run and were restored only by massage.

Respiratory changes. The minute-volume increase of breathing was considerable, the average curve (fig. 1) shows a progressive increase from 5.65 liters as a normal to an average of 41.33 liters in the last minute of the run. Several cases entirely stopped breathing immediately after the run, one case for as long as four minutes. There seems often to be absolutely no desire to breathe.

Carbon dioxide analyses of the alveolar air, by the Haldane technique of sampling during the run, showed a steady decrease from an average of 5.6 per cent as a normal to 3.2 per cent at the middle of the run and 2.13 per cent at an average time of one minute before the end of the run. Four minutes after the run the percentage was 4.3 and fifteen minutes after it was still only 4.4.

The partial pressure of carbon dioxide fell from an average of 39.9 mm. Hg to 22.9 mm. at the middle of the run and to 15.2 mm. one minute before forced breathing was stopped. Such a decrease in alveolar carbon dioxide is indicative of an equally pronounced fall in the carbon dioxide combining power of the venous blood plasma, according to the studies of Collip and Backus (1920) and of Grant and Goldman (1920).

The arterial blood pressures. The mean arterial pressure curves for all of our experiments are shown in figure. 1. They represent the means of 60 cases. Since the runs varied considerably in length and since there was no standard for determining just what stage of acapnia had been attained at any one time, the mean time of all the runs determined the length of the curve, and means were made of the following points: A preliminary or normal; an average of the first three minutes; the 5th minute; the 7th minute; the second minute before the middle of the run; the middle of the run; the second minute after the middle; the 5th, 4th, 3rd and 2nd minutes before the end; the last minute; and the 1st, 2nd, 3rd, 5th, and 10th minutes after the run.

The curves of the mean arterial pressures indicate that there is normally no regular nor constant arterial blood pressure response by man to the effects of hyperventilation of the lungs. In table 1 appear some of the means and their probable errors. The range of the probable errors shows that, on the whole, fluctuations are not large. Not one of our subjects, in the several experiments on him, reacted constantly in the same manner. In many of our best runs neither of the arterial blood pressures gave evidence of a clear cut change. The systolic pressure showed no change in 18.6 per cent, a rise in 40.7 per cent, and a drop in 40.7 per cent of the runs. The diastolic pressure was unchanged in 20.8 per cent, showed a rise in 56.6 per cent, and a fall in 22.6 per cent of the runs. Out of the 60 runs, a rise of 6 mm. or more was obtained 16 times for the systolic pressure and 22 times for the diastolic pressure. A fall of 6 or more mm. occurred 13 times in the systolic pressure and 7 times in the diastolic pressure. The largest increase in the systolic pressure was 25 mm. and in only 3 runs did it exceed 10 mm. The greatest drop in the systolic pressure was 12 mm. The diastolic pressure changes ranged from +14 to −9 mm.

Taken in their entirety, our experiments support the opinion of Collier, Densham, and Wells that the systolic blood pressure is maintained nearly

constant in man during forced respiration. For all our runs the systolic pressure showed a mean increase of only 2 mm. and the diastolic pressure an increase of 3 mm.

There were no signs of after-effects in either of the arterial blood pressures.

The pulse. The frequency of the heart beat, from an average of about 80 at the beginning, started to accelerate at the fifth minute and showed a progressive rise to 96 at the end (see fig. 1 and table 1). The frequency of the pulse was unchanged in 8.9 per cent, retarded slightly in 10.7 per cent, and accelerated in 80.4 per cent of the runs. The rise in frequency, however, is less than that noted in our carbon dioxide (Schneider and Truesdell, 1922) and anoxemia by rebreathing (Schneider and Truesdell, 1921) experiments. There were no signs of after-effects in the pulse, the mean frequency of the pulse being 79.2 ± 0.98 one minute after the forced breathing.

Capillary blood pressure. Capillary pressure was studied with the Danzer-Hooker micro-capillary tonometer and the averages of the results

TABLE 1

Some of the means of arterial blood pressures and the probable errors

	PRELIMINARY	AV. 1ST 3 MINUTES	MIDDLE MINUTE	3RD MINUTE FROM LAST	2ND MINUTE FROM LAST	LAST MINUTE
Pulse	80.6 ±0.81	79.4 ±1.08	86.3 ±0.84	90.4 ±1.07	92.8 ±1.19	96.0 ±1.48
Systolic pressure	106.2 ±0.80	105.8 ±0.89	105.8 ±0.91	106.5 ±0.85	106.3 ±0.93	108.2 ±1.10
Diastolic pressure	71.0 ±0.58	72.5 ±0.53	73.0 ±0.63	73.4 ±0.62	73.4 ±0.61	74.0 ±0.68

are plotted in figure 2. The heavy horizontal black line represents the average of the preliminary readings and is indicated as zero, the other points represent the average amount of increase from this normal. There were eight different subjects and averages were calculated for the same times as those used for the pulse frequency and the arterial blood pressures of figure 1. The curve of the average capillary pressure shows a small slow steady rise in the pressure of 4.1 mm. Hg during the first 15 minutes of hyperventilation of the lungs, and then a fairly uniform state to the end of the run. An analysis of the individual cases shows that four had practically no change until the last minute or two of the run. In no case was there a drop in capillary pressure. Immediately after the period of forced breathing the pressure continued to rise and was 10.3 mm. above the pre-acapnial average 10 minutes later. This rise after the run was shown in all but two cases.

Venous pressure. This was studied by means of the Hooker device. There were 14 runs on 8 different subjects which have been averaged according to the plan used for the capillary pressure. The curve for the

average changes is plotted in figure 2 and represents the average increase in cubic centimeters H_2O over the average preliminary readings. This curve gives an increase of 1.5 cm. H_2O in the venous blood pressure up to the middle of the run, then a fall which never quite restores it to the normal. After the period of hyperventilation, the venous pressure shows a slight rise throughout a period of 10 minutes.

A study of the individual cases shows that they were, in general, fairly uniform; eight of the 14 experiments followed the average curve, four showed a rise without the fall, and two showed practically no change

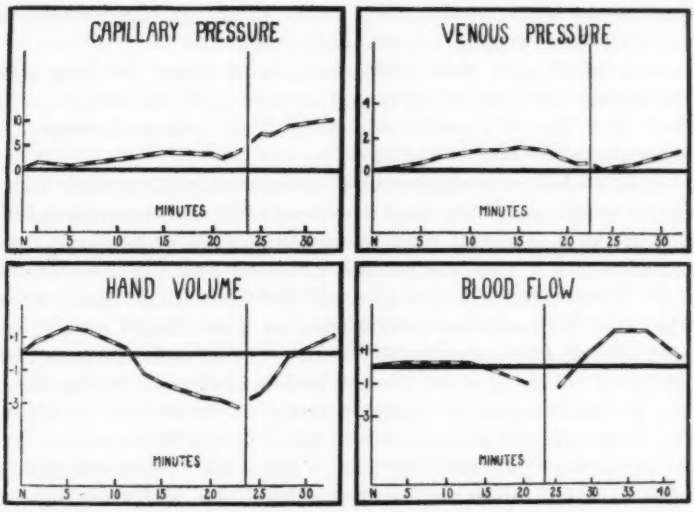

Fig. 2. Curves of the mean response to a gradual voluntary forcing of respiration. Normals have been recorded as *O*, the curves show the amount of change above and below normal. The scale to the left is in millimeters Hg for capillary pressure, in centimeters H_2O for venous pressure, in cubic centimeters for hand volume, and in grams of blood per 100 cc. hand volume for blood flow.

during the run. There were six experiments in which the last reading during the period of hyperventilation was lower than the first or normal reading, the fall bringing it below the average curve. In one of these the venous pressure fell from a pre-acapnial level of 7.4 cm. to 4.1 cm. In the other three cases the fall below normal ranged from 1.5 to 2.9 cm.

In a study of six healthy men, Henderson, Prince, and Haggard (1918) found, by the tilting-board method of determining venous pressure, that this pressure invariably fell below the pre-experiment level as the result of 2 or 3 minutes of forced breathing. They did not determine the pressure

during the period of hyperventilation. Our results are more in agreement with those obtained by Dale and Evans (1922) on cats and rabbits, in which they found the effects of forced breathing variable, with the change in venous pressure never great no matter what its direction.

Hand volume. The hand volume determinations were made by means of a hand plethysmograph. In figure 2 we have plotted the average variations from the normal for 10 forced breathing experiments on 8 different subjects. The points of the curve were established in the same way as those employed for the arterial blood pressure curves. There occurs at first, when the breathing is only slightly augmented, a slight increase in the hand volume; but, as the breathing becomes decidedly excessive, the hand volume falls off moderately.

The individual cases show three instances in which the hand volume did not change, and one in which it increased 5 cc. per 100 cc. of hand volume. For the six cases in which the hand volume decreased, the shrinkage ranged between 4 and 12 cc.

Taken as a whole, our experiments indicate that, while in some individuals hyperventilation of the lungs is without effect on the peripheral blood vessels, in the majority of cases there occurs a vasoconstriction of the peripheral vessels. This result is not in accord with the observations of Dale and Evans on anesthetized cats and rabbits in which rapid excessive ventilation of the lungs was accompanied by a swelling of organs; but is in harmony with an observation of Nahum reported by Henderson, Prince, and Haggard (1918) in which "forced breathing induced during the succeeding period of apnea a distinct decrease in the volume of the arm, smaller pulsations, and a diminution of blood flow of 50 per cent."

In our subjects in whom the hand volume was decreased by hyperventilation of the lungs the normal volume was afterward somewhat slowly restored. In some cases the volume for a time overcompensated. The normal hand volume was usually restored within from 5 to 15 minutes.

Blood flow through the hand. This was determined by Stewart's hand-calorimeter method. There are 11 runs on 10 subjects from which the curve given in figure 2 was prepared. In the curve the average differences from the normal are plotted at the following points: normal, first five minute period, five minute period previous to the middle of the run, middle five minutes, five minute period after the middle, last five minute period, and the five five-minute periods following the run. This average curve shows the flow of blood to be maintained just above normal until directly after the middle of the run, when the flow gradually retards. This finding corresponds with the experience of Stewart (1911) who reported that 4 or 5 minutes of forced breathing reduced the blood flow through the hand approximately 25 per cent.

All of our subjects, except the one who showed the increase in hand

volume, conform well to the average curve. The exceptional subject in two experiments gave a 50 per cent increase in the blood flow. The retardation for the rest of the subjects ranged from 5 to 20 per cent.

After the period of hyperventilation, there is an increase in the blood flow for 10 or 15 minutes which carries it decidedly above normal. Following this the flow slowly returns to normal.

Discussion. In summing up our observations, we recognize that our method of inducing acapnia has differed from that employed by other investigators. The earlier experimenters with animals and with man have induced an excessively rapid removal of carbon dioxide from the body by suddenly and enormously increasing the ventilation of the lungs. Our subjects voluntarily gradually increased their breathing as follows: at the start they breathed 5.65 liters; 5th minute, 7.67; 10th minute, 16.0; 15th minute, 21.1; 20th minute, 32.9; and 22nd minute, 41.33 liters. By this gradual method of forcing the breathing, the body is given ample opportunity to make compensatory adjustments should there be a tendency for hyperventilation of the lungs to depress the circulation.

Our data, taken as a whole, indicate that the effect of voluntary forced breathing on the circulation of man is not constant or definitely predictable. Not one of the circulatory factors studied by us has responded the same in all subjects, and sometimes an individual has reacted differently in the several experiments in which he participated. While the effects of hyperventilation are, therefore, to some extent variable; the changes, whatever they may be, are never great.

So far as our evidence goes it would seem definitely to indicate that in man, during voluntary hyperventilation of the lungs, the arterial blood pressure is usually adequately maintained by compensatory constriction of peripheral vessels, as has been claimed by Collier, Densham, and Wells (1927). That shunting of blood from the periphery does occur is evidenced by the reduction in the hand volume and in the flow of blood through the hand that occurred in the majority of the experiments.

That the venous reservoir was not seriously depleted is indicated by the fact that, in the majority of cases, the arterial blood pressures were either unchanged or slightly elevated; and that in the 40 per cent of the cases which showed a fall this was moderate, the greatest being only 12 mm. The venous blood pressure remained approximately normal in 57 per cent of all cases, and in those in which it went subnormal no negative pressures were recorded.

An increased frequency of heart beat, which was observed in 80 per cent of all cases, no doubt serves as a compensatory response to reductions in the venous return to the heart. This probably would maintain the arterial blood pressure in spite of some pooling of blood in the splanchnic vessels and a reduction in the venous pressure.

SUMMARY

A gradual increase in the minute-volume of breathing from an average of 5.65 to 41.33 liters, during a period of 22 minutes, lowered the alveolar partial pressure of carbon dioxide to 15.2 mm. Hg.

The means of the arterial blood pressures for a group of 60 experiments were not affected by hyperventilation. Approximately 40 per cent of all cases experienced a moderate rise and another 40 per cent a moderate fall in the systolic pressure, while 56.6 per cent had a moderate rise and 22.6 per cent a moderate fall in the diastolic pressure.

The pulse frequency was accelerated in 80, unchanged in 9, and retarded in 11 per cent of the experiments.

The capillary blood pressure slightly rose.

As a rule, the venous blood pressure increased during the first 15 minutes of hyperventilation and then gradually diminished. In approximately 43 per cent of all cases the venous pressure fell below the individual's average pressure.

The volume of the hand usually increased at first and later decreased until it was moderately subnormal.

The blood flow through the hand was practically unaffected for more than the first half of the period of hyperventilation and then was retarded as much as 5 to 20 per cent.

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A NOTE ON THE RELATION OF SALIVARY SECRETION TO THE OXYGEN TENSION OF THE INSPIRED AIR

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Barcroft and his co-workers (1901, 1912b and 1916) have shown that secretion of the submaxillary gland is accompanied by an increase in the rate of metabolism of the gland. The question arises whether the gland requires an immediate supply of oxygen in order to pour out saliva or the additional oxygen consumption is evidence for an immediate "reconstruction" process. If the secretion in response to stimulation is dependent upon the immediate, extra oxygen consumption for its energy supply, then a diminution in the arterial oxygen tension should have an immediate effect upon the secretion of the gland. The following experiments were undertaken with that point in view. Unfortunately, the experiments had to be discontinued without carrying out in detail the plan of attack. None the less, the results obtained, it is felt, are of sufficient interest to warrant their publication.

EXPERIMENTAL PROCEDURE. Dogs, weighing from 15 to 25 kgm., were anesthetized with amytal by either subcutaneous or intraperitoneal injection, or both. The dosage was 0.09 gram per kilo, of an almost neutral solution. As soon as the animal was sufficiently under the anesthetic, a tracheal cannula was inserted. Then one Wharton's duct was exposed, and cannulated at the point just distal to where it loops under the lingual nerve. Immediately thereafter the chorda tympani was found; the lingual nerve severed on either side of the point where the chorda fibres leave the common sheath, and the chorda very carefully dissected free for about an inch in length. In most of the dogs this was then repeated for the other duct and nerve.

In all but the first animal, the drops were permitted to fall from a glass tube, the end of which had been drawn out, into a collecting vessel. As the drops fell from this tube, one of us would make contact with a key which would close a circuit, activating a signal magnet writing on a long paper kymograph. The secretory rate was never so fast that one person could not record the secretion from both glands simultaneously.

The chorda tympani was stimulated, in all dogs after the second, using platinum electrodes sealed into a piece of glass tubing of narrow bore, and

about three-quarters of an inch in length. The nerve, free at the end distal to the gland, was easily drawn over the electrodes without stretching. A Harvard inductorium, with a six volt storage battery in the primary,

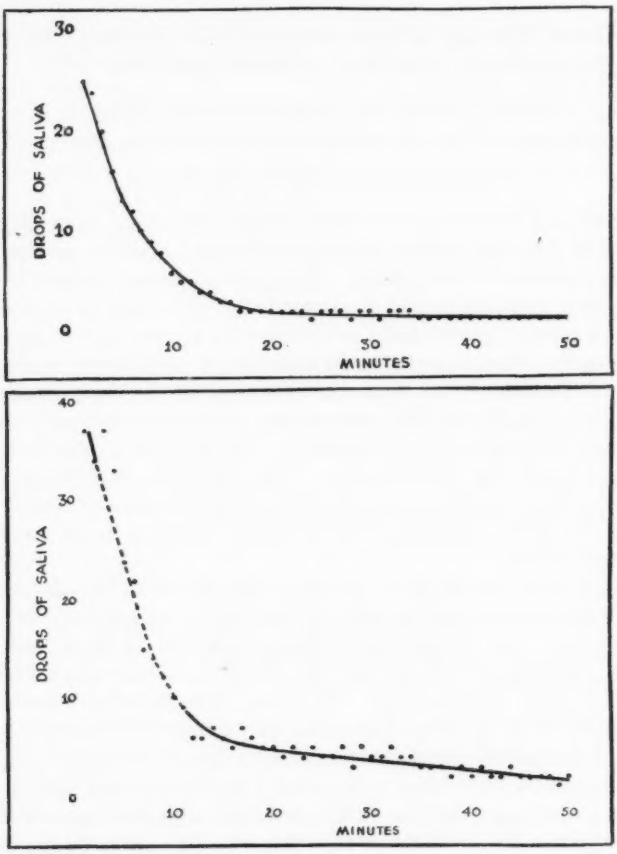

Fig. 1. Upper curve, dog 4, gland previously unstimulated. Lower curve, dog 5, gland previously unstimulated. Solid line, dog breathing room air. Dash line, dog breathing 10 per cent oxygen.

was used for stimulation. A signal magnet was placed in series to indicate the duration of stimulation. Time was recorded with an Ingersoll Timer.

Injections of pilocarpine were made with an hypodermic syringe into either the femoral or saphenous vein. The solution was of 1 per cent strength.

The gas mixtures were made up by diluting room air with hydrogen or nitrogen from a tank of the compressed gas. The mixtures were made up in the bell of a Tissot spirometer, from which the animal breathed, using flutter valves wasting the expired air to the room. While breathing air, the dog breathed through the same set of valves. There was no noticeable effect upon the animals when the valves were connected with the tracheal cannula.

RESULTS. The results, obtained from eight dogs, readily group themselves into three categories: responses to chorda stimulation; responses

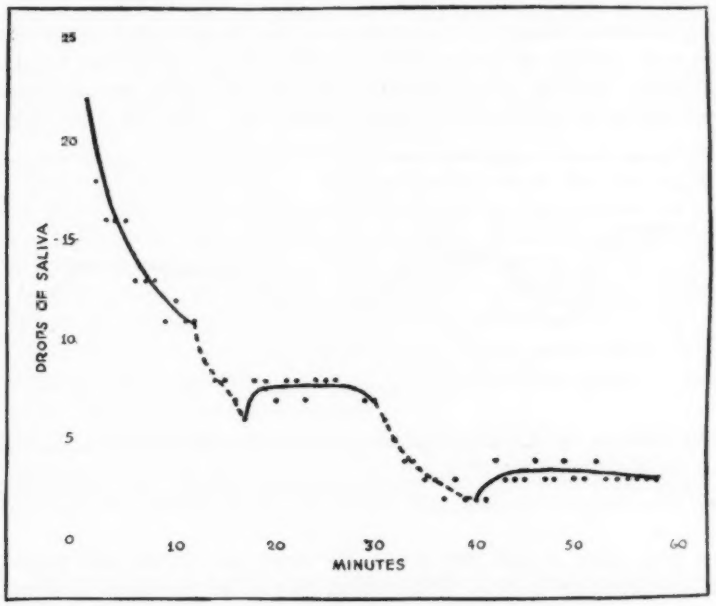

Fig. 2. Dog 2, right gland, following second injection of pilocarpine, 1.5 cc. Solid line, dog breathing room air. Dash line, dog breathing 10 per cent oxygen.

to pilocarpine stimulation; and effects obtainable when the gland was secreting at a steady rate after the first, large response to pilocarpine had worn off.

The administration of the gas with diminished oxygen tension produced a marked cyanosis. Respiration was increased greatly. The diminished alveolar oxygen tension had, however, no noticeable effect on the rate of salivary secretion in response to chorda tympani stimulation. The graphs in figure 1 are typical of the response to chorda stimulation when the dogs were breathing room air and when they were breathing air diluted with

nitrogen (or hydrogen). There is no apparent difference between the two curves.

When the stimulus was changed to pilocarpine, there was a marked difference in the response of the gland when the dog was breathing air, and when breathing the diminished oxygen gas mixture. Figure 2 illustrates this. The dips in the curve occurred during the periods the animal was breathing the depleted air. The recovery on returning to air is as marked as the drop in secretion in response to the lowered oxygen tension. Such a dip in the curve, following pilocarpine, was always produced by diminishing seriously the oxygen tension of the inspired air.

Even more striking were the responses of the gland when it was secreting at a slow, steady rate in response to pilocarpine. Following the huge, immediate response to pilocarpine injection, the gland would continue to excrete for several hours at a slow, steady rate. Any changes in secre-

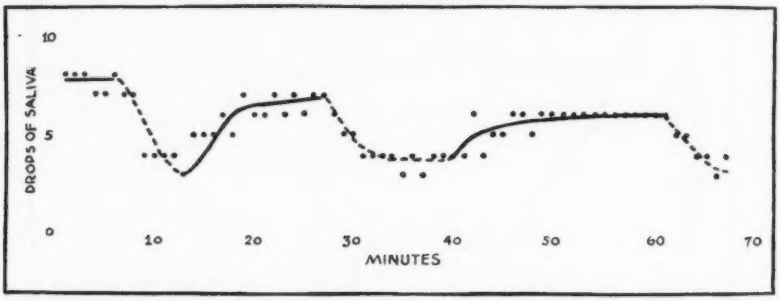

Fig. 3. Dog 6, right gland, one hour after injection of 0.5 cc. 1 per cent pilocarpine. Solid line, dog breathing room air. Dash line, dog breathing 7 per cent oxygen.

tion rate, even though not absolutely large, are during this period of considerable significance. Diminishing the oxygen tension of the inspired air during such steady secretion produced drops in the secretory rate of 50 per cent or more, with immediate recovery upon the readministration of room air. Figure 3 is typical of such results. It is quite evident that there is a difference in the effect of diminished oxygen tension in the alveolar air upon the rate of secretion of the submaxillary gland of the dog depending upon the stimulus to secretion.

Discussion. The meaning of this difference is not at all clear. Studies of the blood flow through the gland, and of the arterial and venous oxygen contents, during the periods of diminished alveolar oxygen tensions would undoubtedly throw light upon the question. Such experiments were planned, but time did not permit of their being carried out. It seems unreasonable, none the less, to believe that the difference can be entirely

ascribed to a change in blood flow through the gland produced by one or the other method of stimulation. Barcroft (1912a) has demonstrated that change in blood flow, *per se*, does not affect submaxillary secretion.

It is suggested that there is a difference in the mode of action of pilocarpine and chorda tympani stimulation. V. E. Henderson (1922) reported that atropine had a differential action in inhibition of pilocarpine and parasympathetic stimulation of the bladder. He found that atropine more completely inhibited the action of pilocarpine than it did that of the chorda. On the other hand, Cannon and Cattell (1916) have shown that atropine completely inhibits the electrical response normally accompanying chorda stimulation.

MacKay (1928) showed that histamine, following pilocarpine, no longer gave the usual increase in blood flow through the submaxillary gland, but on the contrary, a decrease. She suggests that this may be caused by a "true reversal of function brought about by pilocarpine." It is possible that anoxemia produces such a decrease in the blood flow through the gland, and a simultaneous decrease in secretion, in the presence of pilocarpine. Her table 5 illustrates this point well. In this connection might be mentioned the old suggestion of A. P. Mathews (1901) that pilocarpine acts by increasing the rate of oxidation. If so, it might follow that decreasing the oxygen supply would decrease the action of pilocarpine. It is only fair to state that Verzár (1912) showed that oxygen consumption in the submaxillary gland was independent of the arterial oxygen tension. This, however, was in the resting gland.

The question whether the salivary gland depends upon an immediate oxygen supply for its energy seems, for the present, to resolve itself into a question of the type of stimulus employed.

SUMMARY

1. The effect of diminishing the oxygen tension of the inspired air upon the rate of submaxillary secretion in the dog was studied.

2. The response, during anoxemia, was unchanged when the gland was stimulated through the chorda tympani.

3. There was a marked decrease in the secretion rate during anoxemia when the stimulus was pilocarpine.

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ADDENDUM

Since the writing of this paper, there has been called to our attention the paper of Eddy in the April number of this Journal. Our results confirm his in that he also found that decreasing the oxygen content of the inspired air decreased the rate of secretion of the pilocarpinized gland. Furthermore, he has shown that this decrease is independent of the rate of blood flow through the gland.

STUDIES ON THE PHYSIOLOGY OF SECRETIN

I. On the Preparation and Isolation

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A number of workers have attempted to isolate secretin. Numerous properties of various secretin preparations have been ascribed to the hormone. Briefly they are: Augmentation of general metabolism—Aeblous and Soula (1920); stimulation of the hematopoietic tissue—Eddy and Downs (1917–18–21–24), and King (1926); mobilization of carbohydrate—Eddy and Downs (ibid.); causes hypoglycemia—Santos (1925), Zunz and La Barre (1928), and Penau and Simonnet (1925); increases formation of urine; increases flow of bile; augments secretion of pancreatic juice; a fraction separated from secretin preparations causes the gall bladder to contract—Ivy et al (1928). Finally, J. Mellanby (1928) announced the isolation of secretin and described the properties as follows: It is a polypeptid in character; has no effect on blood sugar; increases tone of smooth muscle; has no vasodilatins but causes local vasomotor response in pancreas; has no effect on respiratory system or voluntary muscle; has no effect on the secretion of urine and augments the flow of bile.

It did not seem reasonable to us that secretin could possess such a variety of physiological activities.

We have prepared secretin preparations from the duodenum of the dog which are of higher activity and state of purity, as judged by lack of effects other than pancreatic secretion, than any heretofore reported and confirmed. However, our method has not been always successful in that about 40 per cent of the preparations have not been of the uniform activity reported below. This in part we believe is due to the somewhat complicated nature of the process.

The duodenum of the dog is removed and at once washed with cold water to remove debris, etc., from the mucosa. One end is clamped and the duodenum filled with a solution containing 100 cc. concentrated HCl and 50 cc. H_2SO_4 per 20 liters of water. After thirty minutes' extraction the filtrate is clarified by centrifugation. To the clear filtrate are added 25 grams of solid NaCl per 100 cc. and thoroughly stirred for 20 minutes.

The precipitate which is centrifuged off is essentially that described by Weaver, Luckhardt and Koch (1926). It contains little or no vasodilatin and is a potent secretagogue. This precipitate is dissolved in the original volume of 0.5 per cent H_2SO_4 (50°C.), the insoluble fraction is centrifuged off and discarded. The filtrate is precipitated by Ivy's method of adding solid trichloracetic acid to 5 per cent.

The precipitate is allowed to settle and finally centrifuged at high speed. The filtrate is discarded. The precipitate is washed into a beaker with three volumes of absolute alcohol and thoroughly triturated. An equal volume of acetone is then added and vigorously stirred. For each 100 cc. of the mixture 500 cc. of ether is then added and violently stirred until the supernatant liquid separates clear. The precipitate is collected and dried in vacuo at 40°C. This crude secretin is highly active but possesses also 1, cholecystokinin; 2, hypoglycemic substances; 3, oxytocic substances; 4, protein-like substances; 5, gray color.

Two hundred and fifty milligrams of this crude secretin are thoroughly triturated with 25 cc. of 90 per cent alcohol containing one drop of H_2SO_4 per 250 cc. On centrifuging a pale yellow solution is obtained which is combined with a second extract prepared in the same manner.

Residue contains in the main, cholecystokinin, a small amount of vasodilatins and the substances causing hypoglycemia together with unclassified impurities.

Filtrate contains in the main secretin, usually contains a trace of cholecystokinin, together with some unclassified impurities, but no hypoglycemic substances. To this filtrate is added $\frac{1}{20}$ volume of 1.0 per cent alcoholic brucine. The precipitate is collected after one hour in the ice box. It is virtually secretin free. One-fortieth volume of pyridine is then aded to the filtrate. If a precipitate forms it is separated and discarded. The filtrate is precipitated by one volume of acetone and five volumes of ether. The precipitate is centrifuged off after one hour in the ice box and washed five times with one part acetone and five parts ether. It is then dried in vacuo. This product is almost white and highly active.

This product is powdered and placed in a small modified Soxhlet extractor and extracted with methyl alcohol (about 99 per cent) under reduced pressure for thirty minutes to one hour. In our experience two results may be obtained here. A white precipitate may or may not flocculate out of the alcohol. Usually it is of low activity, however, it *may* contain as much secretin as the soluble portion and should be tested. The filtrate is precipitated in the ice box for one hour with 1 volume of acetone and five volumes of ether, centrifuged and washed with ether and finally dried in vacuo. This preparation is nearly always white and of very high activity possessing the following properties:

1. Doses of 0.02 to 0.10 mgm. per kilo will in a 10 kgm. dog under barbi-

tal, chloralose, or with a pancreatic fistula cause the secretion of 6 to 10 cc. of pancreatic juice in 20 minutes; the rate of secretion being faster and the duration of secretion being shorter than with more impure preparations.

2. Huge doses, 1.0 mgm. per kilo, cause no vasomotor changes, local or general.

3. Huge doses, 1 mgm. per kilo, cause no changes in blood sugar in normal or diabetic dogs whether anesthetized or unanesthetized.

4. Most preparations give a feeble biuret reaction.

5. Our product is cholecystokinin free.

6. No oxytocic properties in concentrations of 1:500,000.

These properties have been determined simultaneously with the same preparations. If 0.10 mgm. per kilo would not cause the secretion of pancreatic juice mentioned above the product was discarded.

In view of the fact that Mellanby had announced the isolation of secretin, we attempted to prepare the product as described by him. We followed his directions on six occasions and at no time did we obtain a product of great activity. This has been the result obtained by Ivy and also by Zunz and LaBarre (personal communications). Zunz and LaBarre (1928) obtained from Doctor Mellanby some of his product and showed it contained not only secretin but also vasodilatin and the hypoglycemic substances as tested in the chloralosed dogs.

We have confirmed Ivy et al as to the presence, action and solubility of cholecystokinin.

From our crude product we have separated the hypoglycemic substances from cholecystokinin and secretin. One such preparation in doses of 10 mgm. intravenously lowered a diabetic dog's blood sugar from 330 mgm. per cent to 200 mgm. per cent in two hours, and a normal dog's blood sugar from 110 mgm. per cent to 63 mgm. per cent in the same time. This substance is not insulin because it is not destroyed by peptic or tryptic activity as is insulin. Nor does it necessarily mobilize insulin for the depancreatized dog reacts to its injection.

In a later paper we shall give a chemical analysis of our various products and protocols showing their physiological properties.

We do not believe our product to be entirely free from impurities; however, we do believe it to be nearly so, certainly it contains no impurity which is physiologically active as shown by the tests used by us.

The author has taken advantage of the advice and criticisms of Dr. A. J. Carlson and Dr. A. B. Luckhardt to whom he is justly grateful.

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ANEMIA IN JAUNDICE

II. The Formation of Hemoglobin in Experimental Obstructive Jaundice[1]

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Although anemia has long been noted in cases of jaundice, both in man (3, 6) and in animals (9), very little has been done to throw any light on its cause. Clinically, anemia occurs frequently in cases of jaundice, whether it is due to obstruction of the common bile-duct by carcinoma of the pancreas or biliary passages, to stone in the common bile-duct, or to intrahepatic disease (4). In experimental animals, ligation of the common bile-duct commonly is followed by a gradual fall in the level of hemoglobin, whereas the curves for serum bilirubin and for bile salts also show a decrease in their values following a preliminary rise (9, 11).

Although an abundance of evidence has been accumulated regarding the origin of bilirubin from disintegrated hemoglobin, it is by no means certain that the conversion of hemoglobin into bilirubin is a quantitative one. Whipple and Hooper have demonstrated that this transformation is modified by the presence of anemia in the experimental animal. The question is closely associated with the problem of metabolism of pigment in general, and much of the recent work has been directed toward learning the sites of formation of bilirubin.

At least two problems remain unsettled. One problem is concerned with the cause of the anemia observed in clinical and experimental types of jaundice. The other deals with the effect of anemia on the amount of bilirubin formed after obstruction of the common bile-duct, and, conversely, with the effect of obstruction on the amount of hemoglobin being regenerated. The work already mentioned showed that both problems were in some way related to each other.

A definite solution of these problems, however, could not be given be-

[1] The data presented in this paper are taken from a thesis submitted by F. M. Jordan to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Medicine, 1929.

cause of the inadequacy of experimental methods. The recent work of Whipple and Robscheit-Robbins showed the important influence of dietary factors on the amount of hemoglobin being regenerated. The method employed by them was admirably suited to the study of regeneration of blood in chronic anemia, and we were able to adapt this method to the study of the problem at hand.

We sought to obtain direct data on the following three points: 1, the effect of diet on the rate of formation of hemoglobin and the change in that rate produced in obstructive jaundice; 2, the modification of this process produced by different degrees of anemia, with consequent variation in the stimulus to regeneration, and 3, the relation of changes in the metabolism of hemoglobin to the degree of bilirubinemia which developed after biliary obstruction.

EXPERIMENTAL PROCEDURES. Whipple and Robscheit-Robbins showed that the different foodstuffs varied greatly in their effect on the regeneration of hemoglobin by the dog. The feeding of some foodstuffs, such as liver, was associated with maximal regeneration of hemoglobin and erythrocytes. Other materials, such as those comprising their basal ration, completely satisfied the requirements of the animal for food and yet permitted only minimal regeneration of hemoglobin and erythrocytes. In these experiments a basal ration of low regenerative power, modelled after that of Whipple and Robscheit-Robbins, was used. It contained the following: cod-liver oil, 50 grams; salmon, 100 grams; tomato juice, 100 grams; bread, 200 grams, and milk, 300 cc. The substitution of beef liver for salmon in this basal ration sufficed to change the character of the diet completely, so far as regeneration of hemoglobin is concerned, and to convert the basal ration to one on which maximal regeneration of hemoglobin was possible. So modified, the diet contained the following: cod-liver oil, 25 grams; beef liver, 100 grams; tomato juice, 50 grams; milk, 300 cc., and glucose, 100 grams.

The stimulus to regeneration was varied by changing the level of hemoglobin in the blood. This was effected by withdrawing large amounts of blood, several times, until the predetermined level had been reached. During the period of employment of each diet, the hemoglobin of one set of dogs was maintained at a level of 6 to 7 grams for each 100 cc. of blood. This low level presumably gave a maximal stimulus for regeneration. The other series of animals was maintained at a level of 10 to 11 grams of hemoglobin for each 100 cc. of blood. Such a level is sufficiently lower than normal to permit of accurate measurement of the hemoglobin regenerated, but near enough to the normal level to result in only a moderate stimulus to regeneration of blood. Thus, four groups of animals were used, depending on the type of diet and the level of hemoglobin employed in each group, as follows: Animals in group 1 (tables 1 and 2) received a diet low

TABLE 1

Results in group 1 (dog 1), with a low level of hemoglobin and a diet low in regenerative power

DATE	WEIGHT, KGM.	SERUM BILIRUBIN, MGM. FOR EACH 100 CC.	MEAN HEMOGLOBIN, GRAMS FOR EACH 100 CC. OF BLOOD	ERYTHROCYTES BY HEMATOCRIT, PER CENT	PLASMA VOLUME, CC.	WHOLE BLOOD VOLUME, CC.	HEMOGLOBIN			
							Total, grams	Grams for each kgm. of body weight	Total removed, grams	Grams removed for each kgm. of body weight
11/28–12/ 4	12.57		11.5	30	630	900	103.5	8.2	64.5	5.1
12/ 5–11	11.45		8.3	26	495	670	55.6	4.8	22.3	1.9
12/12–19	10.94		8.05	25	740	986	79.3	7.3	20.0	1.9
12/19	Operation									
12/20–26	9.84	0.3	6.7				66.1	6.5	19.9	2.2
12/27– 1/ 3	8.75	1.5	6.2	24	520	694	43.0	4.9	1.9	0.2
1/ 4–10	8.16	2.4	6.0				41.6	5.1	0.9	0.1
1/11–17	7.57	3.6	7.05	25	470	625	44.1	5.8	3.0	0.4
1/17	Died									

TABLE 2

Results in group 1 (dog 2, control), with a low level of hemoglobin and a diet low in regenerative power

DATE	WEIGHT, KGM.	SERUM BILIRUBIN, MGM. FOR EACH 100 CC.	MEAN HEMOGLOBIN, GRAMS FOR EACH 100 CC. OF BLOOD	ERYTHROCYTES BY HEMATOCRIT, PER CENT	PLASMA VOLUME, CC.	WHOLE BLOOD VOLUME, CC.	HEMOGLOBIN			
							Total, grams	Grams for each kgm. of body weight	Total removed, grams	Grams removed for each kgm. of body weight
11/28–12/4	9.3		11.5	28	471	656	75.4	7.6	61.2	6.2
12/ 5–11	9.3		7.1	24	569	749	53.2	5.7	18.3	1.97
12/12–18	9.1		7.4	33	602	902	66.7	7.3	20.5	2.2
12/19–25	9.2		7.7						15.2	
12/26– 1/ 1	9.5		7.58	25	546	729	53.5	5.6	15.3	2.9
1/ 2– 3	9.3		7.0						11.7	1.2
1/ 9–15	9.07		7.0	29	553	782	54.7	6.8	2.2	0.2
1/16–22	8.43		7.5	25	568	755	56.6	6.7	14.9	1.77
1/23–29	8.1		7.3	26	548	742	54.2	6.7	14.3	1.8
1/30– 2/ 5	7.8		6.5						15.9	2.0
2/ 2	Operation									
2/ 6–12	7.18	2.6	6.7	23	424	543	36.4	5.7	3.6	0.5
2/13–19	7.2	2.2	6.6				35.8	4.9	0.6	
2/20–26	6.25	2.1	6.3	28	368	510	32.2	5.15	2.6	4.2
2/27– 3/ 3	6.05	1.5	6.2	21	423	535	33.2	5.5	3.0	0.5
3/ 4	Died									

in regenerative power and a low level of hemoglobin was employed; animals in group 2 (tables 3 and 4) received a diet high in regenerative power and a low level of hemoglobin was employed; animals in group 3 (tables 5 and 6) received a diet low in regenerative power and a high level of hemoglobin was employed; animals in group 4 (tables 7 and 8) received a diet high in regenerative power and a high level of hemoglobin was employed.

The hemoglobin was maintained at the desired level of anemia by repeated bleedings at frequent intervals. The changes in the volume of blood and in the hemoglobin were followed and the amount of blood to be removed at each bleeding was estimated thereby. A record was kept at these bleedings of the amount of hemoglobin, in grams, removed, as an index of the amount being regenerated. The volume of blood was determined by the method of Keith, Rowntree and Geraghty. The determinations of hemoglobin by the acid-hematin method were made by using the permanent standard solution described by Osgood and Haskins. The average of all the determinations made each week is indicated as the mean hemoglobin, whereas the total hemoglobin is the product of the mean hemoglobin multiplied by the total volume of blood.

When the amount of hemoglobin being regenerated from week to week was constant, biliary obstruction was produced by ligation of the common bile-duct. The operations were all performed under ether anesthesia and with the use of aseptic technic. While one animal from each group was operated on the other was used as a control, and after several more weeks the animal which had been used as a control was also operated on. There were, consequently, two experiments in each group. The jaundice following the ligation of the common bile-duct was studied by frequent determinations of serum bilirubin, using the Thannhauser and Andersen modification of the method of van den Bergh.

EXPERIMENTAL RESULTS. *Results in group 1.* Dog 1 was given a diet poor in regenerative power and the level of hemoglobin was maintained at 6 to 7 grams for each 100 cc. A total of about 20 grams of hemoglobin was regenerated each week before operation. Following ligation of the common bile-duct, three interesting points were brought out: 1, practically no blood was regenerated, the amount of hemoglobin regenerated varying from 0.9 to 3.0 grams each week; 2, there was a great reduction in the total volume of hemoglobin, and 3, the greatest rise in serum bilirubin was only 3.6 mgm. for each 100 cc. at the end of one month (table 1).

Dog 2 was used as a control for the preceding experiment and later was operated on. Prior to operation, the total amount of hemoglobin regenerated averaged about 17 grams a week, with a slight falling off in later weeks. The total volume of hemoglobin remained relatively constant. Following the operation there was almost no regeneration of hemoglobin, the amount regenerated varying from 0.6 to 3.6 grams with a great re-

duction in the total volume of hemoglobin, whereas the greatest rise in serum bilirubin was only 2.6 mgm. for each 100 cc. (table 2).

Results in group 2. Dog 3 was given a diet high in regenerative power and its hemoglobin was maintained at a low level. The amount of hemoglobin formed prior to operation varied from 12.7 to 32.4 grams a week, averaging about 25 grams a week. Following the operation, there was a great decrease in the amount of blood being regenerated during the first week, due, in part, to difficulties in the animal's taking the diet for the first few days after operation. During the next two weeks, however, the original level was reëstablished. Jaundice became more marked, as indicated by the rise in serum bilirubin to 7.5 mgm. for each 100 cc.; there was a slight fall in serum bilirubin just before death. The volume of

TABLE 3

Results in group 2 (dog 3), with a low level of hemoglobin and a diet high in regenerative power

DATE	WEIGHT, KGM.	SERUM BILIRUBIN, MGM. FOR EACH 100 CC.	MEAN HEMOGLOBIN, GRAMS FOR EACH 100 CC. OF BLOOD	ERYTHROCYTES BY HEMATOCRIT, PER CENT	PLASMA VOLUME, CC.	WHOLE BLOOD VOLUME, CC.	HEMOGLOBIN			
							Total, grams	Grams for each kgm. of body weight	Total removed, grams	Grams removed for each kgm. of body weight
1/24–31	8.7		7.8						28.4	
2/ 1– 7			8.2						32.4	
2/ 8–14	8.6		6.8	21	642	814	55.4	6.4	12.7	1.5
2/16	Operation									
2/15–21		0.4	6.7						2.2	
2/22–28	8.35	7.5	7.8	25	490	654	51.0	6.1	15.9	1.9
2/29– 3/ 6	10.05	4.6	7.4	34	476	722	53.4	5.3	24.5	2.4
3/ 8	Died									

hemoglobin remained practically constant throughout. Another dog used as a control and kept under similar conditions did not show change in the rate of regeneration up to the time of operation.

Dog 4 was given the same diet and its hemoglobin was maintained at the same level. Before operation the amount of hemoglobin regenerated averaged about 11 grams each week. Following the operation there were wide variations in the amount of hemoglobin regenerated, but the average was about 10 grams each week. In some cases, there was a "carry-over" to the next week, so that an average must be taken of the amounts of hemoglobin removed during these two weeks. There was a moderate fall in the total volume of hemoglobin, while the serum bilirubin rose to 5.2 mgm. for each 100 cc.

Results in group 3. Dog 5 was given a diet low in regenerative power and the level of hemoglobin was maintained between 10 and 11 grams for each 100 cc. Since the level of hemoglobin was higher than that in the experiments in group 1, the stimulus for regeneration was less, and the amount of hemoglobin formed while the dog was receiving the same diet was consequently decreased. The total amount of hemoglobin formed each week before operation averaged about 4 grams with a slight carry-over during the second week after the level of hemoglobin had been established. Following the operation, the regeneration of hemoglobin remained nearly the same, or slightly less when expressed as the fall in the total volume of hemoglobin or as the amount of hemoglobin for each kilogram of body weight. The highest value for serum bilirubin was 4.3 mgm. for each 100 cc. In order to determine whether the same response in regeneration would be obtained as had occurred in the previous experiments in group 1, four weeks after operation enough blood was removed to reduce the level of hemoglobin from 11 to 6 grams for each 100 cc.; meanwhile the diet was kept constant. The conditions were now those of group 1, with a low level of hemoglobin and a diet poor in regenerative power. As in experiments 1 and 2, there followed almost complete inhibition of formation of hemoglobin, with a pronounced fall in the total volume of hemoglobin. However, a striking difference was noted in the degree of jaundice, the serum bilirubin gradually rising from 3.9 to 13.5 mgm. for each 100 cc.

Dog 6 was given the same diet and its hemoglobin was maintained at the same high level. The total amount of hemoglobin formed before operation averaged about 3 grams each week. Following the operation, the amount regenerated remained at almost the same level, with a fall just before death. During the first four weeks following operation the highest level of serum bilirubin reached was 3.8 mgm. for each 100 cc. During the last two weeks, due to the condition of the animal, there was a fall in the level of hemoglobin, approximating conditions in group 1, and coincidently with this the level of serum bilirubin rose to 7.7 mgm. for each 100 cc. This result is comparable with that noted in dog 5, in which an induced lowering of the level of hemoglobin, without any change in the diet or rate of regeneration of hemoglobin, resulted in a gradual rise in the level of serum bilirubin to 13.5 mgm. for each 100 cc. In dog 6 the reduction in the level of hemoglobin was spontaneous and was due to the condition of the animal, but here again a gradual rise in the level of serum bilirubin occurred.

Results in group 4. Dog 7 was given a diet high in regenerative power and its hemoglobin was maintained at a high level. Prior to operation the total amount of regenerated hemoglobin varied from 1.1 to 6.9 grams a week, with an average of about 3 grams a week. Following the operation the total amount of hemoglobin regenerated varied from 2.6 to 7.1 grams, with an average of 4.5 grams a week, while the total volume of hemoglobin

remained nearly constant. The greatest rise in serum bilirubin was 7.5 mgm. for each 100 cc. at the end of five weeks following ligation.

Dog 8 was given the same diet and its hemoglobin was maintained at the same high level. Prior to operation a total of about 20 grams of hemoglobin was regenerated each week. Following the operation the regenerative level varied from 14.3 to 18.3 grams a week. The serum bilirubin gradually rose to 7.7 mgm. for each 100 cc. at the end of six weeks. The animal then refused to take food and its condition became poor; there was a gradual loss of weight. This established the conditions of the

TABLE 4

Results in group 2 (dog 4), with a low level of hemoglobin and a diet high in regenerative power

DATE	WEIGHT, KGM.	SERUM BILIRUBIN, MGM. FOR EACH 100 CC.	MEAN HEMOGLOBIN, GRAMS FOR EACH 100 CC. OF BLOOD	ERYTHROCYTES BY HEMATOCRIT, PER CENT	PLASMA VOLUME, CC.	WHOLE BLOOD VOLUME, CC.	HEMOGLOBIN			
							Total, grams	Grams for each kgm. of body weight	Total removed, grams	Grams removed for each kgm. of body weight
12/14–20	15.35		7.8	28	994	1,380	107.6	7.0	19.8	1.3
12/21–28	15.6		7.3	27	1,050	1,430	104.2	6.7	21.4	1.4
12/29– 1/ 4			7.9				113.0	7.3		
1/ 5–11			8.5				121.5	7.8	17.3	1.1
1/12–18	15.3		7.0	26	1,006	1,353	94.6	6.2	13.5	0.8
1/19–25	15.1		7.0	24	990	1,301	91.0	6.0		
1/26–31	15.0		6.8	22	976	1,250	85.0	5.7	3.6	0.2
1/31	Operation									
2/ 1– 7	14.8	2.5	7.5	24	950	1,215	91.0	6.2	1.5	0.1
2/ 8–14	14.0	3.0	8.2	22	930	1,200	93.5	6.7	20.8	1.5
2/15–21	13.5	3.7	6.8	20	910	1,150	78.3	5.8	2.5	0.2
2/22–28	12.9	4.4	7.4	23	878	1,140	84.4	6.6	2.9	0.2
3/ 1– 7	12.5	5.2	7.9	27	788	1,080	85.5	6.8	21.7	1.7
3/ 8–14	Died									

experiments in group 3, namely, a high level of hemoglobin and a diet low in regenerative power. A marked fall in the level of regenerated hemoglobin occurred, and, at the same time, a fall in the curve for serum bilirubin.

COMMENT. These experiments confirm the work of Whipple and his co-workers regarding the effect of dietary factors on the regeneration of hemoglobin, and also emphasize several points which they mentioned. They stressed the importance of careful selection of the animals, and the relation of exercise, sunlight, and clean conditions of living. Whipple also mentioned the fact that there are individual variations in the reactions shown by different animals, and this was borne out by our work.

The operation consisted only of ligation of the common bile-duct; the gall bladder was not removed. The influence of the gall bladder in delaying the development of bilirubinemia has been stressed by Mann and Bollman, and by Snell, Greene and Rowntree. Following the operation anorexia and vomiting developed in many of the animals and in such circumstances recourse was to feeding by tube.

All of the animals tended to lose weight gradually, after obstruction to the common bile-duct, in spite of a diet adequate in protein and calories;

TABLE 5

Results in group 3 (dog 5), with a high level of hemoglobin and a diet low in regenerative power

DATE	WEIGHT, KGM.	SERUM BILIRUBIN, MGM. FOR EACH 100 CC.	MEAN HEMOGLOBIN, GRAMS FOR EACH 100 CC. OF BLOOD	ERYTHROCYTES BY HEMATOCRIT, PER CENT	PLASMA VOLUME, CC.	WHOLE BLOOD VOLUME, CC.	HEMOGLOBIN			
							Total, grams	Grams for each kgm. of body weight	Total removed, grams	Grams removed for each kgm. of body weight
2/ 1– 7			15.2						79.8	
2/ 8–14	10.57		11.8	33	598	894	105.5	10.5	4.4	0.4
2/15–21			12.4				111.0	10.5	19.8	1.8
2/22–28	10.18		10.7	34	622	942	101.0	9.8	3.2	0.3
3/ 1	Operation									
2/29– 3/ 6	9.7	0.3	10.7	23	523	684	73.2	7.5	5.4	0.5
3/ 7–13	9.32	2.2	11.5	33	479	715	82.2	8.8	6.8	0.7
3/14–20	9.15	4.3	11.0				78.6	8.7	2.9	0.3
3/21–27	8.95	3.9	11.4	30	565	810	92.3	9.3	4.7	0.5
3/28– 4/ 2	Hemoglobin reduced to low level									
4/ 2– 9	7.43	4.5	7.0	29	512	722	50.5	6.8	2.5	0.3
4/10–16		6.3	6.6				47.7	7.2	1.3	0.2
4/17–23	6.3	6.9	6.8	24	518	682	43.0	6.8	2.7	0.4
4/24–30		7.3	6.7				45.7	6.8	2.0	0.3
5/ 1– 7		8.5	6.4				43.6	6.8	1.6	0.2
5/ 8–14		13.5	7.1				48.4			
5/15	Died									

this possibly may be due to the effect of excluding bile from the digestive tract.

The stools were watched for evidences of bile, diarrhea and blood, and in all cases, after death, necropsy was done to be sure that the bile had been effectually excluded from the gastro-intestinal tract and that there were no complicating factors.

In a previous study of 347 clinical cases of obstructive and intrahepatic jaundice it was shown that anemia occurred frequently in jaundice due to

any cause, independently of infection or of loss of blood, and that there was no correlation between the degree of anemia and either the duration or the intensity of jaundice (4).

When the common bile-duct in dogs is ligated there is a rapid rise to a maximum in the amount of serum bilirubin and of bile salts; this is followed by a gradual fall to a comparatively low level (11). At the same time, there is a progressive fall in the hemoglobin, and anemia is thus produced which usually remains stationary at a moderately low level. The same effect has been noted in dogs with biliary fistulas.

Theoretically, at least, the anemia may be due to excessive hemolysis, to deficient formation of blood, or to a combination of both factors.

The question of excessive hemolysis is frequently studied by determining the amount of bile excreted by dogs with biliary fistulas. This method is obviously not applicable to the study of obstructive jaundice. It is well known that bile salts are hemolytic, and the suggestion is commonly made that the anemia occurring in obstructive jaundice is due to the hemolytic action of these salts. Several facts are against this view. A point of great significance is that the concentration of bile salts necessary to produce hemolysis, as determined in vitro, does not occur in clinical or experimental cases of jaundice. Moreover, as Snell, Greene and Rowntree showed, following a preliminary rise in the bile salts, after obstruction of the common bile-duct in dogs, there is a gradual fall in their level in the blood. Brakefield and Schmidt demonstrated also that the curve of urinary excretion of the bile acids in obstructive jaundice shows similar changes. It would be expected, therefore, if bile salts were the cause of the anemia, that anemia would appear very soon after the onset of jaundice, when the concentration of bile salts is greatest. In clinical cases the anemia is variable in intensity and may occur early or late. Experimentally the anemia increases coincidently with a fall in the concentration of bile salts in the blood. Furthermore, blood serum has a pronounced protective action against hemolytic agents. Bayer, and also Sellards, reported that hemolysis by bile salts was prevented when sufficient serum was added to the mixture of bile salts and erythrocytes. Moreover, Brakefield and Schmidt found that the excretion of bilirubin in the urine decreases following obstruction; since there is associated increasing anemia, one would expect increased elimination of bilirubin if the anemia were due to hemolysis.

Rous and Drury suggested that there was a definite relationship between changes in bilirubinemia and variations in the percentage of hemoglobin, although they recognized the fact that when the circulating hemoglobin of dogs with biliary obstruction was depleted by bleeding there was sometimes a more than corresponding reduction in the bilirubinemia. Whipple and Hooper showed that under certain conditions, such as anemia, in-

jected hemoglobin may not be quantitatively eliminated as bile pigment, and that it may aid in the recovery from anemia. These experimental results suggested a relationship between the degree of bilirubinemia and the metabolism of hemoglobin. The recent work of Whipple and Robscheit-Robbins indicates definitely that dietary changes influence the amount of hemoglobin being formed, but there are few direct data demonstrating any relationship between the degree of bilirubinemia and variations in the rate of formation of hemoglobin.

TABLE 6

Results in group 3 (dog 6, control), with a high level of hemoglobin and a diet low in regenerative power

DATE	WEIGHT, KGM.	SERUM BILIRUBIN, MGM. FOR EACH 100 CC.	MEAN HEMOGLOBIN, GRAMS FOR EACH 100 CC. OF BLOOD	ERYTHROCYTES BY HEMATOCRIT, PER CENT	PLASMA VOLUME, CC.	WHOLE BLOOD VOLUME, CC.	HEMOGLOBIN			
							Total, grams	Grams for each kgm. of body weight	Total removed, grams	Grams removed for each kgm. of body weight
2/ 8–15	14.2		11.8	31	908	1,301	153.5	10.8	16.5	1.2
2/16–22	12.96		11.2	31	937	1,350	151.2	11.7	1.0	0.07
2/23–29	11.92		10.7	31	967	1,401	150.0	12.6	2.8	0.2
3/ 1– 7	11.55		11.7	30	727	1,040	121.7	10.5	3.5	0.3
3/ 8–14	11.58		11.1	35	707	1,085	120.4	10.4	5.1	0.4
3/15–21	11.67		10.1	40	707	1,187	120.0	10.4	26.2	2.2
3/22–28	11.0		10.1	30	786	1,120	113.1	10.3	4.2	0.4
3/ 9– 4/ 4			9.5				106.4	9.7	1.0	0.09
4/ 5	Operation									
4/ 5–11	12.0	0.6	9.8	27	845	1,156	113.2	9.5	3.0	0.25
4/12–18	11.4	3.6	11.5	30	835	1,200	138.0	12.1	2.3	0.2
4/19–25	10.85	3.8	12.2	34	824	1,245	152.0	14.0	3.7	0.3
4/26– 5/ 2	10.4	2.9	10.6	31	817	1,185	132.0	12.7	3.1	0.3
5/ 3– 9	9.9	6.2	9.6	28	810	1,125	108.0	10.9	1.9	0.2
5/14		7.7	8.1				91.1			
5/15	Died									

With regard to the influence of nutritional factors on the amount of hemoglobin regenerated following ligation of the common bile-duct, the experiments in group 1 show that when dogs are given a diet poor in regenerative power, and their hemoglobin is maintained at a low level, ligation of the common bile-duct results in inhibition of formation of hemoglobin. Essentially the same effect was noted in the experiments in group 3, in which the same diet was used during a period when the hemoglobin of the animal was at a high level. Here, however, the difference was less striking, presumably because of the lesser stimulus to regenera-

tion, with a higher level of hemoglobin. It appeared, then, that the anemia was due to a fault in the formation of blood. If the amount of blood being formed could now be modified by varying the quality of the diet, it would seem reasonable to assume that the cause of the deficient formation of blood was dependent on nutritional factors. Consequently, in the experiments in groups 2 and 4, a diet high in regenerative power was tested at a time when hemoglobin was at low and at high levels. In each case, following ligation of the common bile-duct, it was found that only a slight fall in the total amount of circulating hemoglobin and the

TABLE 7

Results in group 4 (dog 7), with a high level of hemoglobin and a diet high in regenerative power

DATE	WEIGHT, KGM.	SERUM BILIRUBIN, MGM. FOR EACH 100 CC.	MEAN HEMOGLOBIN, GRAMS FOR EACH 100 CC. OF BLOOD	ERYTHROCYTES BY HEMATOCRIT, PER CENT	PLASMA VOLUME, CC.	WHOLE BLOOD VOLUME, CC.	HEMOGLOBIN			
							Total, grams	Grams for each kgm. of body weight	Total removed, grams	Grams removed for each kgm. of body weight
12/12–18	12.35		10.1	34	918	1,390	140.5	11.4	5.4	0.4
12/19–26	12.25		11.1	39	955	1,565	173.8	14.2	28.7	2.3
12/27– 1/ 2			11.0				153.0	12.5	1.1	0.1
1/ 3– 9			10.3				143.0	11.7	2.0	0.2
1/10–16	12.0		11.9	38	875	1,385	165.0	13.8	1.7	0.14
1/17–23	11.9		11.5	37	850	1,280	147.0	12.4	1.2	0.1
1/24–30	11.65		12.7	40	667	1,125	143.0	12.3	6.9	0.6
1/31	Operation									
1/31– 2/ 7	11.5	3.8	12.8	38	712	1,163	149.0	13.0	2.6	0.2
2/ 8–14	11.4	4.0	11.8	39	726	1,175	138.8	12.2	4.1	0.4
2/15–21	11.3	5.3	12.1	37	745	1,190	144.0	12.8	3.9	0.3
2/22–28	11.4	7.2	12.1	38	760	1,225	143.2	12.6	4.8	0.4
3/ 1– 7	10.6	7.5	11.8	36	750	1,175	138.7	13.1	7.1	0.7
3/10	Died									

total amount of hemoglobin regenerated occurred. It would seem, therefore, that in animals which are receiving a diet poor in regenerative power, as in those of groups 1 and 3, ligation of the common bile-duct inhibits regeneration of hemoglobin, whereas animals which are receiving a diet high in regenerative power, as those of groups 2 and 4, are much more resistant to this effect of obstruction.

The nutrition of the animals might be modified either by changes in the diet or by variations in absorption of food from the gastro-intestinal tract. We were able to control the factor of diet. Exclusion of bile from the intestine presumably interferes with absorption and consequently we

could not be certain whether a poor nutritional condition was a direct result of ligation of the common bile-duct or whether it was dependent on some fault in the absorption of food, but the difference noted in the results in the experiments in groups 1 and 3 as compared with those in the experiments in groups 2 and 4 emphasizes the former effect. Although the exclusion of bile may influence the degree of absorption, the diets administered were in all cases adequate in calories but varied in their effect on

TABLE 8

Results in group 4 (dog 8), with a high level of hemoglobin and a diet high in regenerative power

DATE	WEIGHT, KGM.	SERUM BILIRUBIN, MGM. FOR EACH 100 CC.	MEAN HEMOGLOBIN, GRAMS FOR EACH 100 CC. OF BLOOD	ERYTHROCYTES BY HEMATOCRIT, PER CENT	PLASMA VOLUME, CC.	WHOLE BLOOD VOLUME, CC.	HEMOGLOBIN			
							Total, grams	Grams for each kgm. of body weight	Total removed, grams	Grams removed for each kgm. of body weight
3/22–29	13.25		12.5	40	804	1,337	167.5	12.7	46.3	3.4
3/30– 4/ 5	13.2		12.1	40	950	1,584	192.0	14.6	40.5	3.0
4/ 6–12	13.2		11.7	40	868	1,403	164.2	12.4	20.6	1.5
4/13–19	13.2		11.4	37	859	1,346	153.6	11.6	0.6	0.04
4/20–26	13.24		11.2	34	851	1,290	144.6	10.9	2.2	0.16
4/26	Operation									
4/26– 5/ 3		1.3	12.3							
5/ 4–10			12.5						18.3	1.5
5/11–17		5.6	12.2						16.0	1.3
5/18–25	11.45	5.6	12.3	44	584	884	138.0	12.5	15.2	1.3
5/26–31	11.40	6.5	11.1	41	636	1,002	111.2	11.0	0.6	0.05
6/ 1– 7	11.35	5.9	11.9	38	688	1,120	133.2	11.8	15.0	1.3
6/ 8–14	10.80		11.4	33	739	1,120	127.8	11.8	14.3	1.3
6/15–21	10.25	7.7	11.5	29	790	1,120	128.8	12.6	2.2	0.2
6/22–28	10.0	5.4	9.8	29	761	1,082	106.0	10.6		
6/29– 7/ 5	9.75	3.0	10.5	30	732	1,045	109.7	11.3	3.8	0.4
7/ 6–12	9.7	2.6	9.5	30	700	1,000	95.0	9.8	3.0	0.3
7/13–19	9.7	1.8	9.7	30	668	954	93.4	9.6	3.0	0.3
7/20–26	8.7		9.7	30	567	810	78.6	9.1	2.4	0.3
8/ 1	Died									

regeneration of hemoglobin. Consequently, when we mention the result of a good nutritional state we refer especially to the degree of regeneration of hemoglobin as influenced by diet or by the probable specific factor influencing regeneration of blood that is contained in such substances as liver.

In these experiments, the degree of bilirubinemia was observed to vary. When the common bile-duct was ligated in the dogs of groups 1 and 3, only a slight degree of jaundice resulted; the greatest rise in serum

bilirubin was 3.6 mgm. for each 100 cc. In the experiments in group 2, the serum bilirubin rose to a higher level; in dog 3 it reached 7.5 mgm. and in dog 4 it rose to 5.2 mgm. for each 100 cc. The experiments of group 4 also resulted in a greater rise as compared with the results in groups 1 and 3; in dog 8, the serum bilirubin rose to 7.7 mgm. whereas in dog 7 it rose to 7.5 mgm. In the first experiment in group 3 (dog 5), after the results had been observed for several weeks, the level of hemoglobin was quickly reduced, by bleeding, to a low level; thus the conditions of group 1 were established and there then followed a gradual but definite rise in the serum bilirubin from 3.9 to 13.5 mgm. for each 100 cc. The same effect was noted in the second experiment in group 3 (dog 6), in which the level of hemoglobin fell slightly toward the end of the experiment, and at the same time there occurred a gradual rise in the curve of serum bilirubin.

We recognize the variability in different animals in regard to the intensity of jaundice resulting after ligation of the common bile-duct. One must be careful, therefore, in drawing conclusions from a limited number of experiments. Nevertheless the results recorded here seem to indicate that neither the level of hemoglobin nor the total amount of circulating hemoglobin influences the degree of bilirubinemia to any great extent. Essentially the same degree of jaundice resulted in group 1 and in group 3. In both groups the amount of hemoglobin regenerated was very small following operation, whereas the level of hemoglobin and the total amount of circulating hemoglobin were low in group 1, but high in group 3. Under the same conditions, but with an increase in the amount of hemoglobin regenerated, the experiments in groups 2 and 4 resulted in a greater rise in bilirubinemia. It appeared, therefore, that one factor influencing the degree of bilirubinemia was the extent of regeneration of hemoglobin and the consequent activity of the metabolism of pigment, rather than the level of hemoglobin or the total amount of circulating pigment.

A curious effect was noted, however, in the two experiments of group 3, in which the level of hemoglobin of the dogs was kept high and they were given a diet poor in regenerative power. Following the operation there was only a slight rise in the serum bilirubin. In dog 5, the level of hemoglobin was rapidly lowered by bleeding and maintained at a low level. Following this maneuver, without changing the character of the diet, there occurred a rise in the serum bilirubin to 13.5 mgm. In dog 6 the same effect was noted when there occurred a fall in the level of hemoglobin and a decrease in the total amount of circulating hemoglobin as the result of nutritional difficulties and loss of weight. The effect of the lowered level of hemoglobin was an increased stimulus to regeneration, but because of the poor diet, regeneration of hemoglobin could not be accomplished. Apparently, then, an increased stimulus to the hematopoietic tissues, with or without an increase in the amount of hemoglobin regenerated, may influence the degree of bilirubinemia.

SUMMARY AND CONCLUSIONS

The study of the anemia occurring in obstructive jaundice has been fraught with difficulties because of the inadequacy of experimental methods, but improvements in the methods of chemical analysis of the blood and the recent experimental work of Whipple and Robscheit-Robbins in chronic anemia have made such a study possible. By using standardized diets of high and of low regenerative power, and by testing each diet on animals in which the levels of hemoglobin were high and on others in which the levels were low, we were able to study the effect of ligation of the common bile-duct on the amount of hemoglobin regenerated. We were able, also, to obtain direct data on the influence of variations in the level of hemoglobin, the total amount of circulating hemoglobin, and the rate of regeneration on the degree of jaundice resulting after ligation of the common bile-duct. The anemia that occurs in experimental obstructive jaundice seems to be due, in great part, to defective formation of blood. The extent of regeneration of hemoglobin after obstruction of the common bile-duct appears to depend on the diet and the level of hemoglobin. The degree of bilirubinemia seems to vary with the amount of blood being regenerated and with the activity of the metabolism of pigment, rather than with the total amount of circulating hemoglobin or with the level of hemoglobin in the blood.

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FURTHER STUDIES OF THE CHEMICAL REACTIONS UNDERLYING THE COAGULATION OF THE BLOOD. THE ACTIVITY OF LECITHIN

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In a previous study of the coagulation of the blood (1), the investigation of the action of cytozyme and serozyme, which Bordet has described in detail, led to the demonstration of what appeared to be a fundamental chemical reaction between the lipoid and protein substances of the blood in the presence of calcium. By means of the reaction of calcium chloride with the lipoid substance in an alkaline solution it was possible to separate from the crude lipoid extract of heart tissue a fraction containing a substance which possessed coagulative activity. Furthermore, it was possible to obtain this active lipoid substance in a relatively pure form, but with considerable difficulty, and the results of the analyses varied. Nevertheless, sufficient evidence was adduced to suggest that the active lipoid substance had a structure similar to cephalin, in which the organic base, amino ethanol, had been replaced by hydrogen.

The results of previous work on the nature of the lipoid substance associated with the clotting of the blood have pointed to cephalin rather than lecithin; largely on account of the early work of Howell (2) and the more recent work of Gratia and Levene (3). Gratia and Levene prepared pure lecithin and demonstrated that it was only the impure or contaminated product, containing 75 per cent cephalin and 25 per cent lecithin, which was active in the process of clotting. The close relationship of cephalin and lecithin in all these studies and the observations of Gratia and Levene suggest the importance of a further investigation of lecithin and cephalin and of the active substance which was described in our previous report (1).

The first step was the preparation of pure lecithin. An adaptation of the method published by Levene and Rolf (4) was used and the details of the technic are described in another paper (5). A very satisfactory yield of lecithin was obtained from both liver and heart tissue and the analyses indicated very pure products. The analytical results for the liver and heart lecithin used in this study were as follows:

	CARBON	HYDROGEN	NITROGEN	AMINO NITROGEN	PHOSPHORUS
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Liver lecithin	65.18	10.38	1.94	0.005	3.90
Heart lecithin	64.66	11.14	2.06	0.000	3.87

With these lecithins the previous observations of the inactivity of the pure product in the coagulative process were fully confirmed. These lecithins were then subjected to the methods used in the separation of the active lipoid substance from the crude lipoids contained in beef-heart extract. However, in order to bring about the cleavage of the lecithin molecule, to allow the calcium chloride to react with the liberated fraction, heat was necessary. A hydrolyzed fraction of pure lecithin prepared in this way possessed coagulative properties practically equal in degree not only to that of the crude lipoid extract of heart tissue but also to that of the active lipoid substance prepared from heart tissue, as reported in the previous paper (1).

EXPERIMENTAL PART. *Preparation and isolation of the hydrolyzed fraction of lecithin.* One- or two-gram quantities of the pure lecithin, described above, were dissolved in 50 cc. of absolute alcohol, and 250 cc. of distilled water were added. The mixture was made alkaline with ammonia and 100 cc. of normal calcium chloride solution were added. No precipitation occurred at this point whereas the active lipoid substance was precipitated from the crude lipoid material by such treatment. The mixture was then brought slowly to the boiling point during about 30 minutes, which occasioned the gradual deposition of a precipitate. When cool, the precipitate which had formed was removed by centrifugalization and washed twice with absolute alcohol and once with acetone, in the shaking machine and the centrifuge. The precipitate was then dried in air and suspended in 100 cc. of normal hydrochloric acid. This acid suspension was shaken for 10 minutes in the rapid shaker and then transferred to a separatory funnel, extracted with ether and the acid and calcium were removed by washing with distilled water until the wash water gave no test for chlorides with silver nitrate. The ether extract, free from acid, was evaporated to dryness and the residue taken up in alcohol. This alcoholic solution represented a hydrolyzed fraction of lecithin, precipitable by calcium chloride in alkaline solution, from which the calcium had been removed, and thus corresponded to the fraction which represents the active lipoid substance.

The examination of the coagulative properties of the hydrolyzed fraction of lecithin, of pure lecithin, and of crude tissue extract of the heart lipoids possessing high coagulative properties. The hydrolyzed fraction of liver and heart lecithin, together with the pure lecithins and an alcoholic extract of beef-

heart tissue (cytozyme of Bordet) were tested in parallel for coagulative properties.

Two methods of testing the coagulative function were used: first, the test of the accelerating action of the lipoid solutions on the clotting of recalcified oxalated rabbit plasma from which the platelets had been removed; and second, the test for the production of a solution possessing very active and rapid clotting properties by the treatment of a serum possessing little or no coagulative action with the activating lipoid substance. This inactive serum was obtained by the defibrination of recalcified oxalated plasma from which the platelets had been removed as described below. Such a serum is rich in residual fibrinogen and poor in the lipoid substance, which has been removed in large part with the platelets. According to the French school, it is rich in so-called "prothrombin" and in conjunction with "cytozyme" (lipoid) gives rise to very active "thrombin."

REAGENTS. *Oxalated plasma.* In securing the oxalated plasma, a rabbit was anesthetized and a section of the carotid clamped lightly. An incision was then made in the isolated section of the carotid which was washed free of blood with oxalated salt solution. A paraffined cannula was then tied into the vessel. The first few drops of blood were discarded and 27 cc. were then collected in a paraffined tube containing 3 cc. of 1-per-cent sodium oxalate in 0.5-per-cent salt solution. This oxalated blood was centrifugalized at high speed for one hour. The supernatant plasma was then removed with a pipette (bulb) to clean centrifuge tubes and recentrifugalized for one hour. It was then poured into a sterile, stoppered bottle and kept in the cold room until used.

Lipoid solutions. The pure lecithins used and also the active tissue extract (cytozyme of Bordet) were one per-cent solutions in alcohol.

The hydrolyzed fractions of lecithin represented the total alcohol-soluble residue from 1 gram of lecithin, treated as described above, taken up in 10 cc. of alcohol.

The suspensions were prepared by evaporating 1 cc. of each of these alcoholic solutions to dryness on watch crystals and resuspending in 5 cc. of physiological saline.

The calcified saline. The calcified saline was a 0.035-per-cent solution of calcium chloride in physiological saline.

The "prothrombin." A few cubic centimeters of the oxalated plasma, poor in platelets, prepared as described above, were recalcified and defibrinated. The serum was allowed to stand at room temperature overnight to allow for deterioration of so-called thrombin. A serum prepared in this manner has little or no coagulative action by itself but after preliminary treatment with a lipoid solution, possessing the clotting factor, and in the presence of ionized calcium salts, acquires very active coagulative properties.

The dioxalate plasma. This strongly oxalated plasma was prepared by adding 4 volumes of 0.2-per-cent sodium oxalate, in physiological saline, to 1 volume of the oxalated plasma described above.

Protocol 1. Comparison of the coagulative action of liver lecithin, a hydrolyzed fraction of liver lecithin and of a crude lipoid extract of heart tissue (cytozyme Bordet) on an oxalated rabbit plasma poor in platelets

REAGENTS	QUANTITIES						
	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Calcified saline	0.5	0.5	0.5	0.5	0.0	0.0	0.0
Tissue lipoid (cytozyme Bordet)	0.1	0.0	0.0	0.0	0.1	0.0	0.0
Pure liver lecithin	0.0	0.1	0.0	0.0	0.0	0.1	0.0
Hydrolyzed liver lecithin	0.0	0.0	0.1	0.0	0.0	0.0	0.1
Saline	0.0	0.0	0.0	0.1	0.5	0.5	0.5
Oxalated plasma	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Clotting time in water bath at 37°C	20 min. 45 sec.	No coagulation in 15 hrs.	21 min. 55 sec.	No coagulation in 15 hours			

Protocol 2. Comparison of the coagulative action of liver lecithin, a hydrolyzed fraction of liver lecithin and of a crude lipoid extract of heart tissue (cytozyme Bordet) in formation of so-called thrombin

REAGENTS	QUANTITIES								
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Prothrombin solution	0.2	0.2	0.2	0.2	0.0	0.2	0.0	0.0	0.0
Tissue lipoid (cytozyme Bordet)	0.2	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0
Pure liver lecithin	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.2	0.0
Hydrolyzed liver lecithin	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.2
Calcified saline	0.2	0.2	0.2	0.2	0.2	0.0	0.2	0.2	0.2
Saline	0.0	0.0	0.0	0.2	0.4	0.4	0.2	0.2	0.2
In bath at 37°C. 6 minutes									
Dioxalate plasma	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Clotting time in water bath at 37°C	1 min. 5 sec.	No coagulation in 2 hrs.	1 min. 45 sec.	No coagulation in 2 hours					

PROTOCOLS AND RESULTS. In experiment 1, in which the accelerating effect of the hydrolyzed liver lecithin upon the coagulation of recalcified oxalated plasma was tested, the hydrolyzed lecithin, like the crude tissue

extract, coagulated oxalated plasma in about 20 minutes in the presence of calcium chloride. Pure lecithin either alone or in the presence of cal-

Protocol 3. Comparison of the coagulative action of heart lecithin, a hydrolyzed fraction of heart lecithin and of a crude lipoid extract of heart tissue (cytozyme Bordet) on an oxalated rabbit plasma poor in platelets

REAGENTS	QUANTITIES						
	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Calcified saline	0.2	0.2	0.2	0.2	0.0	0.0	0.0
Tissue lipoid (cytozyme Bordet)	0.1	0.0	0.0	0.0	0.1	0.0	0.0
Pure heart lecithin*	0.0	0.1	0.0	0.0	0.0	0.1	0.0
Hydrolyzed heart lecithin	0.0	0.0	0.1	0.0	0.0	0.0	0.1
Saline	0.3	0.3	0.3	0.4	0.5	0.5	0.5
Oxalated rabbit plasma	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Clotting time in water bath at 37°C.	19 min. 42 sec.	No coagulation in 15 hrs.	16 min. 53 sec.	No coagulation in 15 hours			

* Heart lecithin no. 10.

Protocol 4. Comparison of the coagulative action of heart lecithin, a hydrolyzed fraction of heart lecithin and of a crude lipoid extract of heart tissue (cytozyme Bordet) in formation of so-called thrombin

REAGENTS	QUANTITIES								
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Prothrombin solution	0.2	0.2	0.2	0.2	0.0	0.2	0.0	0.0	0.0
Tissue lipoid (cytozyme Bordet)	0.2	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0
Pure heart lecithin	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.2	0.0
Hydrolyzed heart lecithin	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.2
Calcified saline	0.2	0.2	0.2	0.2	0.2	0.0	0.2	0.2	0.2
Saline	0.0	0.0	0.0	0.2	0.4	0.4	0.2	0.2	0.2
In bath 6 minutes at 37°C.									
Dioxalate rabbit plasma	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Clotting time in water bath at 37°C.	1 min. 32 sec.	No coagulation in 2 hrs.	2 min. 19 sec.	No coagulation in 2 hours					

cium chloride, calcium chloride alone, and lipoid solutions alone had no clotting effect in 15 hours.

In experiment 2 the hydrolyzed fraction of pure liver lecithin, like the crude tissue extract, activated the so-called prothrombin solution to bring about clotting of a strongly oxalated plasma in less than 2 minutes, while pure liver lecithin had no effect. The controls likewise remained uncoagulated.

The coagulative action of pure heart lecithin and of its hydrolyzed fraction were tested in a similar manner. The results are shown in protocols 3 and 4. Pure heart lecithin, like pure liver lecithin, possessed no coagulative activity, whereas the hydrolyzed fraction possessed coagulative properties similar to the hydrolyzed liver lecithin and the crude tissue extract (cytozyme of Bordet).

SUMMARY AND CONCLUSIONS

By a process of mild hydrolysis and precipitation in alkaline solution with calcium chloride, a fraction was isolated from pure lecithin which possessed active coagulative properties. The pure lecithin itself had no coagulative action. This hydrolyzed fraction of pure lecithin corresponds very closely both in method of preparation and in coagulative activity with the active lipoid substance previously obtained from the crude acetone insoluble lipoids of beef-heart tissue. In our previous studies we suggested that this active lipoid substance might be the residue of cephalin resulting from the replacement of the base, amino ethanol, with hydrogen, which is a diglyceride phosphoric acid. The results of the present study suggest that a corresponding residue of lecithin and the active lipoid obtained from tissue extract are similar substances.

Further studies of this hydrolyzed fraction of lecithin are in progress in order to compare it with the active lipoid substance previously obtained; also similar studies of cephalin.

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THE INFLUENCE OF BEEF MUSCLE, BEEF LIVER AND LIVER EXTRACT ON THE ANEMIA OF FASTING AND OF RICE DISEASE IN PIGEONS

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In a previous series of studies, a rather close relationship was observed to exist between the changes which develop in pigeons during simple fasting and in rice disease. Thus the terminal depression of body weight, temperature and respiration was quite similar in degree under such conditions and was modified mainly by the acuteness of the inanition. The course of the anemia, i.e., the decrease in red cell counts, hemoglobin values, cell volumes, tendency toward hydremic plethora, as well as the blood sugar changes which developed in fasting and in rice disease also differed only quantitatively and the degree of the changes noted likewise roughly paralleled the course of inanition. The greatest degree of anemia however was observed in rice disease, very probably as a consequence of the more prolonged period of development of inanition, so that the hematopoietic organs became progressively less able with time to compensate for the rate of blood destruction.

The ease with which this rather extensive anemia could be developed suggested that such a dietary regime offered a suitable means for testing the efficacy of various therapeutic measures used for treating secondary anemias, and simultaneously permit a closer study of the causative factor or factors associated with this type of anemia.

The first paper of this projected series of studies (Barlow, 1927), has shown the ineffectiveness of adding loosely bound HCl (betaine HCl) or vitamins A and C in correcting the deficiencies of the rice diet. The daily addition of lactose, magnesium sulphate or mineral oil to the rice diet, however, relieved or prevented the anemia although the course of inanition was not influenced. This favorable action of the cathartics was provisionally attributed to limitation of toxemia and possibly of bacteriemia.

The second study which forms the basis of this report was undertaken as a consequence of the reported beneficial effects of beef and liver in other types of nutritional anemias (Whipple and Hooper, 1918; Minot and

Murphy, 1926; Whipple, 1928; and Hart et al, 1928), with the object of comparing the effectiveness of these agents on blood regeneration in the fasting and rice type of nutritional anemia. Shortly before completion of this study, observations on the influence of beef and liver on the anemia of fasting were reported by Muller, (1927), which parallel a part of the present investigation.

METHODS. Normal adult pigeons were placed on a mixed whole grain (commercial pigeon feed) diet for three to six weeks in order to establish a constant body weight and blood picture under laboratory conditions. Throughout this period, the weights, red cell counts, cell volumes and hemoglobin determinations in grams per 100 ccm. of blood (Newcomer) were obtained weekly. These observations were continued at four to seven day intervals during the succeeding experimental period. In computing changes in color index, it was necessary to establish a standard for the pigeons. A unit of normal pigeon blood contains 95 per cent of the human hemoglobin values by the Newcomer standard. The normal erythrocyte count of pigeon blood is approximately 4,000,000 cells per cubic millimeter. The normal color index was thus represented as:

$$\frac{\text{The estimated Hb. percentage of normal (actually 95) } 100}{\text{Percentage normal R.B.C.} - \frac{x}{4{,}000{,}000}\ (100)} = 1.0$$

FASTING. Normal pigeons of comparable body weights were divided in four groups of 7 to 9 birds each and subjected to a 10 day fast, with access to water. The fast was then terminated by feeding diets consisting either of whole grain, cooked or raw ground beef muscle, cooked or raw ground beef liver, or mixtures of beef muscle and liver; noting the relative degree of weight and blood regeneration which occurred. The feeding was

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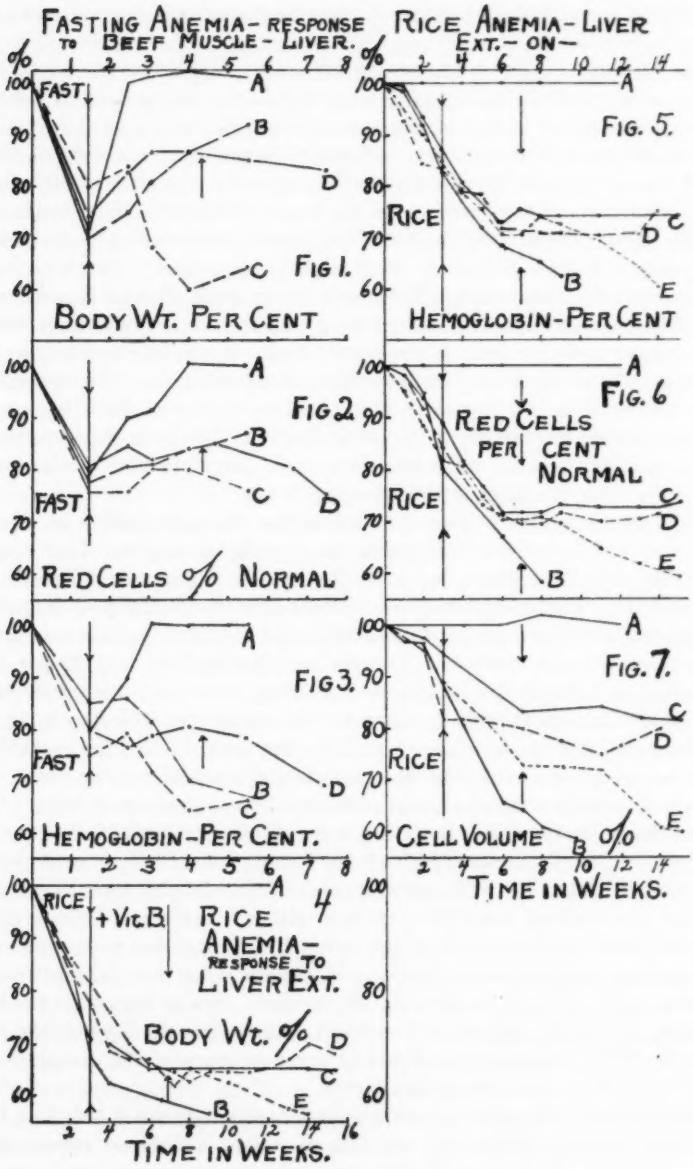
Figs. 1 to 7

Fasting anemia—figs. 1, 2 and 3. Groups A, B, C and D were fasted during the first 10 days. Thereafter group A received whole grain. Group B received cooked or uncooked ground beef muscle. Group C received cooked beef (75 per cent) and liver (25 per cent) from the 10th to 30th and cooked beef (25 per cent) and liver (75 per cent) from the 30th to the 54th day. Group D received cooked or uncooked liver.

Rice anemia. Figs. 4, 5, 6 and 7. Group A received mixed whole grain during the first 6 weeks, grain plus $\frac{1}{10}$ ampoule per kgm. Lilly Liver Ext. from the 6th to 10th, and 1 ampoule per kgm. (plus grain) from the 10th to 12th weeks. Groups B, C, D, and E received polished rice during the first three weeks. Thereafter—Group B received polished rice only. Group C received rice plus Harris Yeast Ext. (150 mgm. daily). Group D received rice plus yeast ext. (150 mgm. daily) from the 3rd to the 7th weeks and $\frac{1}{10}$ ampoule liver ext. per kgm. was added daily from the 7th to 14th weeks. Group E received rice plus yeast ext. (150 mgm. daily) from the 3rd to 7th weeks, and rice plus $\frac{1}{10}$ ampoule per kgm. liver ext. daily from the 7th week to end of experiment.


FASTING ANEMIA- RESPONSE TO BEEF MUSCLE - LIVER.
FAST
A
B
C
D
FIG 1.
BODY WT. PER CENT
FIG 2.
RED CELLS % NORMAL
FIG 3.
HEMOGLOBIN-PER CENT.
RICE
+ VIT. B
RICE ANEMIA- RESPONSE TO LIVER EXT.
BODY WT. %
TIME IN WEEKS.
4
RICE ANEMIA- LIVER EXT. - ON -
FIG. 5.
HEMOGLOBIN-PER CENT
FIG. 6
RED CELLS PER CENT NORMAL
FIG. 7.
CELL VOLUME %
TIME IN WEEKS.

Figs. 1 to 7

continued for sufficient periods of time to permit conclusions as to the efficacy of such diets.

RICE DISEASE. The study of the influence of liver extract no. 343 (a generous supply of which was furnished by the Eli Lilly Research Laboratories) on the anemia of rice disease was made with the object of comparing the effectiveness of the extract on the more severe anemia of this condition with that noted with liver feeding in fasting anemia, and if possible to note any differences in the reactions of the two conditions to liver treatment.

RESULTS. *The influence of grain, beef muscle and liver diets on the weight, cell count and hemoglobin regeneration following a fast.* Figures 1 to 3. *Normal mixed whole grain.* Feeding a whole grain diet at the end of a ten day fast resulted in complete recovery of the body weight level within seven days in 60 per cent of the experiments. Complete recovery in 100 per cent of the experiments was reached on the 11th day. The regeneration of red cells and hemoglobin was definitely slower than the rate of recovery from the fasting weight loss (fig. 1). The hemoglobin returned to normal by the 11th day while the red cell count did not reach normal until the 19th day after feeding was resumed.

Beef muscle. The median curves showing the regeneration of weight, red cells and hemoglobin following the feeding of a ground beef muscle (cooked) diet on terminating a 10 day fast is illustrated in figures 1B, 2B and 3B. The animals had an excellent appetite throughout the period of feeding as pointed out by Miss Muller and ate large quantities of meat. The median weight recovery however was incomplete (only 92 per cent normal) on termination of the meat feeding period, i.e., after 28 days, although the normal body weight was reached by the 18th day in 12 per cent of the series and in 25 per cent by the 28th day on the meat diet. The regeneration of red cells was gradual and reached a value which was 87 per cent of the original normal at termination of the meat feeding. The hemoglobin on the other hand did not regenerate parallel with the red cells but gradually diminished from the fasting value of 85 per cent normal to 74.5 per cent at the 28th day of meat feeding. At this time 25 per cent of the pigeons had hemoglobin values within 5 per cent of the original normal while in 62 per cent of the series, the hemoglobin percentage was 25 or more per cent below normal. The color index was reduced from a normal value of 1 to 0.92 after the 10 day fast. Seven days after the beef feeding began, the index had risen to 1.03 but after 28 days on the beef diet the index diminished to 0.856 as a consequence of the divergence of the erythrocyte and hemoglobin curves.

The dietary value of lean beef muscle for pigeons is much less than that of whole grain as judged by the rate of weight and blood regeneration after fasting. The data of Muller agree with these observations, although in her conclusions she places the emphasis on the more perfect recoveries.

The low dietary value of meat in these experiments is not due to cooking, i.e., destruction of vitamin B, for feeding raw meat which was eaten equally well, did not alter the direction of the curves. Very possibly the deficiency is due to a low vitamin B content of the meat as shown by Hoagland (1929).

Liver feeding. The feeding of a diet consisting of cooked or raw beef liver ad lib. on terminating a 10 day fast is not followed by a satisfactory regeneration from the inanition weight and blood values. The median body weight recovered slightly (increased 4 per cent above the fasting values although this change was probably due to the crop content) during the first 7 days of liver feeding but rapidly decreased thereafter so that at the conclusion of the period of study, i.e., after 28 days, the body weight was 15 per cent less than at the beginning of the liver feeding and 35 per cent of the experiments had ended fatally. The red cell counts and hemoglobin values showed a slight regeneration (4 per cent) which may have been due to other causes, after 7 to 11 days of liver feeding, but they definitely and progressively diminished thereafter to values below the previous fasting levels for these observations (figs. 2 and 3c). The median color index of this series, due to comparable changes in the erythrocytes and hemoglobin was unchanged (0.98) by the 10 day fast. Seven days after liver feeding was begun, the index had risen to 1.04 but after 28 days of liver feeding, the index had a value of 0.895. In so far as the color index changes were concerned, therefore, beef or liver feeding produced comparable changes in spite of the partial weight and blood regeneration noted with the beef diet.

Liver alone is unsatisfactory either as a normal or as a blood regeneration diet after the development of a fasting anemia in pigeons. The data of Muller agree with these observations except that she did not continue the liver feeding sufficiently long to bring out the more serious features of its ultimate inadequacy. This reaction of the birds to liver feeding is in contrast to the reactions of rats under similar experimental conditions (Whitehead and Barlow, 1929). The ineffectiveness of liver as a diet for pigeons is partly explained by the lack of appetite for the liver since the birds voluntarily starve themselves, i.e., the food intake progressively decreases after the first week of feeding; but it must also involve other dietary deficiencies, since forced feeding with large quantities of raw liver does not influence the course of inanition. The deficiency apparently is associated with a lack of vitamin B. The difference in the vitamin requirements of birds and of rats probably explains the divergence of the results obtained under similar conditions with these animals.

Beef and liver feeding. The striking differences in the appetites of the birds for beef muscle and for liver as well as the relative efficiency of such diets for weight and blood regeneration following a fast is illustrated in figures 1, 2 and 3D. The feeding of a mixture of beef (75 per cent) and

liver (25 per cent) following a 10 day fast, resulted during the first 11 days in a weight regeneration from the preceding fasting weight value of 74 per cent normal to 86.5 per cent. No further improvement in weight was noted after an additional 7 days on such a diet. The erythrocyte and hemoglobin values improved gradually and the regeneration with this diet at the 18th day corresponded closely to that noted with beef alone, (figs. 2 and 3B). This diet was then discontinued and a mixture of liver 75 per cent and beef 25 per cent substituted during the succeeding 24 days. The appetite of the birds immediately decreased and at the end of the study a small but definite decrease in body weight was evident. The deficiency of such a diet however was reflected promptly by the development of an anemia which was greater in extent at the end of the 24 day feeding period than that noted following the preliminary 10 day fast.

A diet consisting of beef muscle (75 per cent) and liver (25 per cent) apparently contains sufficient beef to maintain the appetite and to cause a gradual regeneration of the weight and cell counts but not of the hemoglobin level. The substitution of a diet containing 75 per cent liver and only 25 per cent beef however is followed by the development of an inanition curve which is quite comparable to that noted with a liver diet only.

The influence of liver extract on the weight and blood regeneration in rice disease. Figures 4 to 7. Five groups of pigeons were used for this purpose. Group A received a normal diet throughout plus 10 times the human therapeutic dosage of liver extract daily. The maximal human dosage of the extract was taken as 6 ampoules per day, per 60 kgm. body weight. The corresponding pigeon dosage was computed on this per kgm. basis, the powder was sufficiently diluted with talc and administered in gelatin capsules. Group B (control) received polished rice during an 8 week period. Group C received polished rice from the 1st to 3rd and rice plus a 65 per cent maintainence dosage of Harris yeast extract (150 mgm. daily per pigeon) in order to prolong and to more carefully control the course and degree of inanition. Groups D and E received polished rice during the first 3 weeks and rice plus 150 mgm. yeast extract daily from the 3rd to 7th weeks. From the 7th to the 14th weeks, group D received in addition a therapeutic dosage of liver extract. With group E, however, the yeast extract was discontinued after the 7th week and a therapeutic dose of liver extract given instead. During the last 10 days, the liver extract administered to groups D and E was increased to 10 times the usual therapeutic dosage.

The daily administration of therapeutic doses ($\frac{1}{10}$ ampoule per kgm. body weight) of the Minot and Murphy Liver Extract (Lilly Liver Extract no. 343) over a seven week period did not influence in any way the weights or blood pictures of pigeons receiving either a normal or a polished rice diet. Similar negative results were obtained with daily dosages, corresponding

to ten times the human dose (per kgm. body weight basis) over a 10 day period.

The ineffectiveness of a liver diet in fasting and of liver extract in rice disease thus definitely differentiates the nutritional anemia of such conditions from that of a pernicious type, but further emphasizes a certain parallelism of the changes in fasting and in rice disease and their relation to inanition.

CONCLUSIONS

The influence of grain, cooked beef muscle, cooked liver, and mixtures of beef muscle and liver on the weight and blood regeneration of pigeons following a 10 day fast was observed. The efficiency of these diets from greatest to least was whole grain, beef muscle, 75 per cent beef and 25 per cent liver, 25 per cent beef and 75 per cent liver, and liver.

The inadequacy of the liver diet and to a lesser extent of the beef is probably due to its low vitamin B content.

The anemia of rice disease in pigeons was not influenced in any manner by the addition of daily doses of Lilly Liver Extract no. 343 corresponding to the human therapeutic maximum daily dose, to the polished rice diet. Similar results were obtained with birds on rice and also on normal diets with daily dosages corresponding to 10 times the human therapeutic dose.

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THE EFFECT OF THE SPLANCHNICS UPON GLOMERULAR BLOOD FLOW IN THE FROG'S KIDNEY[1]

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Claude Bernard, in 1859, was the first investigator to publish his findings on the nerve supply of the kidney. He was a contemporary of the members of the great, early triumverate of kidney physiologists, Bowman, Ludwig and Isaacs, whose publications in 1842, 1844 and 1857, respectively, can be said to have begun the modern era of kidney physiology.

Bernard first proceeded to study the nerves running to the kidney. He sectioned the splanchnics in dogs and rabbits and noted that the secretion of urine was increased. When the peripheral ends of these nerves were stimulated with a galvanic current, he observed that the secretion of urine stopped, and was not resumed during the period of excitation. On the other hand, he observed that section of the vagus and stimulation of its peripheral end produced no change in the outflow of urine. In one case of stimulation of the central end of the cut vagus, however, he did obtain a decrease in the urine output. Following this he observed that section of the spinal cord stopped the secretion of urine, but if artificial respiration was administered, the urine flow began again. And lastly, in a curarized animal, first without respiratory movements, and then with artificial respiration, he obtained the same results.

From his experiments and the available data, Bernard drew up his theory of kidney function. In the first place, he thought that the kidney acted as a simple filter. For this the blood pressure and the other normal physiologic requirements were necessary, but he believed that they played a passive rôle, whereas the nerve supply played an active rôle. This active rôle of the nerves was their control over the capillary circulation, similar to that which he describes as occurring in the liver. He believed that all of the functionally important capillaries were not active all the

[1] A summary of the findings was reported to the Minnesota Branch of the Society for Experimental Biology and Medicine and published in the Proc. Soc. Exper. Biol and Med., 1929, xxvi, 792.

The experiments reported herein form part of the thesis on "Contributions to the Physiology of the Kidney" submitted in May, 1929, to the Graduate School of the University of Minnesota, by the author, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

time or, in other words, that there was a functional intermittency. We can see, therefore, that Bernard had some idea of intermittency of glomerular blood flow, which recently has been observed and beautifully described for the frog's kidney by Richards (1922) and has been indirectly demonstrated on the mammalian kidney by Khanolkar (1922), Hayman and Starr (1925) and Moore and Lukianoff (1929).

From the time of Bernard down to the present, section of the splanchnics has been repeatedly observed to increase the flow of urine. In animals, results identical with those of Bernard, have been reported by Eckard (1869), Knoll (1872), Klecki (1879), Vogt (1898), Grek (1912), Rhode and Ellinger (1913), Jungmann and Meyer (1913), Quinby (1916), Bellido (1917), Marshall and Kolls (1919–20), Marshall and Crane (1922), Milliken and Karr (1925), Ellinger and Hirt (1925), Hirt (1926), and others. Ustimowitsch (1870) reported the same results with section of all the renal nerves. Peyrani (1881) and Schwarz (1900) alone reported decreased urine flow following splanchnic section.

To the results obtained by splanchnic section can also be added the findings of investigators who have reported favorable results and increased urine output from transplanted kidneys, principally in the case of autogenous transplants. Here again the results have to do with kidneys minus splanchnic as well as other nerves. These favorable results have been reported by Carrel and Guthrie (1905–10), Carrel (1908), Capelle (1908), Unger (1908), Borst and Enderlin (1909), Villard and Tavenier (1910), Villard and Perrin (1913), Lobenhoffer (1913), Zaaijer (1914), Ingebrigsten (1914), Quinby (1916–17), Dederer (1918–20), Boeminghaus (1923), Avramovici (1924), Williamson (1923, 1926), Holloway (1926), Ibuka (1926), and others; and in most cases increased urine output has been reported for at least some days following the operative procedure. These investigators are also of the opinion that life is compatible with the presence of denervated kidney or kidneys, and cite from their experiments that animals are capable of living a normal life with a single denervated kidney up to six years (Zaaijer (1914)). Bellido (1917), on the other hand, in animals with the renal nerves divided, found increased urine output following this surgical procedure, but reported that the animals died in several weeks, with a gradual suppression of urine and coma. Koennecke (1923) reported that denervated kidneys put out a decreased amount of urine and further stated that denervated kidneys were not able to carry on a function greater than normal.

Another well-known function of the splanchnic nerve is its effect on kidney volume and blood flow. Roy and Cohnheim (1883) were the first to study this problem with satisfactory results. They used, for the first time, an oncometer (so called by Roy (1880)) to study changes in kidney volume. From their experiments with this instrument they obtained a

description of kidney volume in the normal animal and in animals following various measures such as asphyxia and stimulation of nerves. Simple section of the splanchnics did not produce a definite increase in kidney volume as they had anticipated, but stimulation of the peripheral end of the cut splanchnic produced a marked contraction of the kidney, as well as a definite increase in blood pressure. Similar types of contraction were also produced by stimulation of the central ends of various cut nerves such as the sciatic and vagus (reflex contractions). Lastly, using a denervated kidney, they found that the various measures employed above, produced no change in kidney volume unless there was a corresponding change in blood pressure; that is to say, the kidney increased in size with a rise of blood pressure and decreased in size with a fall of blood pressure.

The work of Roy and Cohnheim was confirmed and greatly elaborated upon by Bradford (1889). This investigator repeated the anatomical studies of Gaskell (1886) and modified them by stimulating the nerves electrically. Working on dogs primarily, and also on cats and rabbits, Bradford was able to demonstrate not only vaso-constriction within the kidney and resulting shrinkage, but also vaso-dilatation and expansion of the kidney. For determining kidney effects, a Roy oncometer was used. The electrical stimulus was obtained from a DuBois Reymond coil with Helmholtz's modification, and the intensity of the current was such that it was just perceptible to the tongue. Beginning with the posterior roots of the spinal cord, he stimulated the cut peripheral ends, one by one with a rate of five to fifty shocks per second, and obtained marked construction of the kidney. He obtained these effects with roots beginning with the sixth dorsal and terminating in the second lumbar, the most marked effect being obtained from stimulation of the roots from the tenth to the thirteenth dorsals. Now using a similar stimulus, but with a rate of one or two per second, on these same cut roots, he obtained dilatation of the kidney. These effects again were most marked from stimulation of the roots from the tenth to the thirteenth dorsals. Applying these same two rates of stimulation to the splanchnic nerves he again obtained the same results. Applying them now to the peripheral end of the divided vagus, Bradford, like Roy and Cohnheim, obtained no effect on the kidney. Now stimulating the central ends of cut sensory nerves such as the sciatic, vagus and intercostal, he again confirms Roy and Cohnheim in that he produced reflex contraction of the kidney. Finally, Bradford stimulated the central ends of the cut posterior roots. From this he obtained a great variation of effects made up of contraction and dilatation of the kidney and various mixtures of the two. Thus it can be seen that as far as blood vessel effects are concerned, the kidney is an organ well supplied with vaso-constrictors, and to a lesser extend, with vaso-dilators.

Another important link was added to the chain when it was shown by Burton-Opitz and Lucas (1908) that section of the splanchnics increased the blood flow through the kidneys, whereas stimulation of the splanchnics decreased the blood flow. Further, in 1916, Burton-Opitz showed that the right and left splanchnics were distributed to the right and left kidneys, respectively.

Now if we pause and look back, we can say, like Cushny (1926), that from the preponderance of evidence, the following facts can be said to have been established: that section of the splanchnics, which thus removes their influence, results in an increase in urine output, whereas stimulation of their cut peripheral ends, which thus leads to an increase in their function, results in a decrease in urine output. We can then associate with this the findings that section of the splanchnics increases the blood flow through the kidney, whereas stimulation of their cut peripheral ends decreases the renal blood flow. In the light of modern tendency of kidney physiology, the question immediately arises: What part is played by the glomerulus in these variations of function?

Richards and Schmidt (1924), and Richards (1925), state that stimulation of the sympathetic fibers to the kidney and stimulation of the central ends of a cut sensory nerve such as the sciatic, increases the intermittency of blood flow through the glomeruli. They also include a statement to the effect that intermittency of glomerular flow is to be seen when even the brain and spinal cord are destroyed. Referring to the effect of influences which may produce vaso-dilatation and vaso-constriction in the kidney, they state: "Among the former are to be counted section of the sympathetic nerve supply to the kidney. . . ," and "Among the latter are to be included afferent nerve stimulation, direct stimulation of the sympathetic nerve supply to the kidney . . .;" but they offer no experimental confirmation of these statements.

The effect of electrical stimulation of the sympathetic nerves to the kidney contained in the splanchnics, as well as the effect of stimulation of the central ends of cut sensory nerves such as the sciatic and vagus, upon the glomerular blood flow in large and small fields of the kidneys of Rana pipiens and Rana catesbiana, was therefore studied. For electrical stimuli the Harvard induction coil with one dry cell was used. The secondary coil was set at 12 cm. and at this point, when the electrodes were applied to the tongue, they would just produce a sensation, as described by Bradford (1889). In some experiments the secondary coil was moved in closer to the primary, but in all experiments described, there was no spreading of the current to other structures in the frog's body. For application to a nerve, a Sherrington electrode was used, as advised by Dr. F. H. Scott of the department of Physiology. The electrode was mounted on the frog board with a movable clamp so that it would remain stationary while the

current was being applied, and thus the glomeruli could be observed during this procedure.

The frogs used for these experiments were anesthetized by means of a lymph sac injection of isopral (tri-chlor-isopropyl alcohol), 1 mgm. per 10 to 12 grams of frog. A 5 per cent solution of isopral was made with 20 per cent alcohol and 80 per cent water. Following the injection the frog was allowed to remain unmolested for from thirty to forty minutes, before use. This anesthetic has given much better results than urethane; that is, the frogs appear to be in better condition as regards respiratory activity,

TABLE 1

Effect of electrical stimulation of splanchnics

FROG NUMBER	BEFORE STIMULATION		SECONDARY COIL SET AT	DURATION OF STIMULATION	END OF STIMULATION		FIVE MINUTES LATER	
	Act.	Inact.			Act.	Inact.	Act.	Inact.
			cm.	*minutes*				
1	68	0	12	6	43	10	68	0
	Twenty-five minutes' rest							
	68	0	12	6	49	8	68	0
2	89	0	12	6	32	22	89	0
3	52	0	12	4	42	10	52	0
	Thirty-five minutes' rest							
	52	0	4	2	22	28	52	0
4	62	0	5	1½	32	10	52	0
5	22	0	10	5	10	22	22	0

Act. = Glomeruli showing blood flow. Inact. = Glomeruli showing no blood flow.

Upper and lower splanchnic trunks.

heart rate and blood pressure, than those anesthetized with urethane. Blood pressure readings taken on frogs by the method reported by Bieter and Scott (1928) have indicated that there is less depression from isopral than from urethane. The surgical procedures and preparation of the kidneys for microscopic observation were the same as those described by Bieter and Hirschfelder (1924). Frogs in the best condition only, were used, and the experimental procedures were performed as rapidly as possible, because a gradual fall of blood pressure in frogs prepared as above, has been noticed over a period of several hours, with a corresponding decrease in glomerular activity. Where counts of normal active and inactive glomeruli were made, the counts were repeated several times at five or ten minute intervals

so as to be sure that no variation was occurring within the animal that might vary the results of the experiments.

When a frog was prepared as above, and a kidney (in these experiments generally the left was used) was brought into focus with the lower power of an ordinary microscope, and the splanchnics of the same side were stimulated, it could be seen, as Richards and Schmidt (1924) have reported, that the blood flow through many of the glomeruli stopped. If counts of active glomeruli along the lateral border of the ventral surface of the kidney were made before and during stimulation, it could be observed, as is shown in table 1, that the number of active glomeruli were reduced by about fifty per cent. The better the general condition of the frog, the more marked has been the effect observed; example: frog no. 2, table 1. All of

TABLE 2

Effect of electrical stimulation of splanchnics on small fields of glomeruli

EXPERIMENT NUMBER	ACTIVE	SECONDARY COIL SET AT 10 CM.	RESULT OF STIMULATION
		seconds	
1	5	15	5 stopped, resumed flow in 60–80 seconds
2	3	10	3 stopped, resumed flow in 30–50 seconds
3	5	15	4 stopped, resumed flow in 90–120 seconds
4	2	10	2 stopped, resumed flow in 2 minutes
5	3	15	3 stopped, resumed flow in 30–60 seconds
6	3	5	3 stopped, resumed flow in 30–40 seconds
7	2	10	2 stopped, resumed flow in 30–60 seconds
		Repeated five times in thirty minutes	
8	4	10	3 stopped, resumed flow in 30–50 seconds
9	7	15	4 stopped, resumed flow in 40–70 seconds
10	5	15	1 stopped, resumed flow in 30 seconds

Active = glomeruli showing blood flow.

the glomeruli did not remain inactive throughout the period of stimulation; some resumed flow and in others the flow stopped, so that the total number of active and inactive glomeruli showed about the same relationship during the stimulation. Stimulation of the upper splanchnic trunk alone has not been quite as effective as stimulation of the lower and smaller trunk; therefore, in these experiments, stimulation of both trunks was employed. Table 2 shows the effect of splanchnic stimulation upon small fields of glomeruli. It can be seen that when attention is focused upon one small field, the percentage of arrest is far greater; thus in six out of ten recorded experiments, on different frogs, all of the visible active glomeruli stopped. When the stimulus is discontinued, the inactive glomeruli resume flow within two minutes. In the case of some glomeruli it has been observed that only after four or five repeated short periods of stimulation, with a

rest period between each, has it been possible to stop blood flow. On the other hand, other glomeruli have been stopped two, four, six, eight and even ten times, by so many successive stimulations, and at the end of this time, there was no apparent difference in the manner of their response and return to normal. It has also been noticed during splanchnic stimulation that some fields of kidney tissue become paler than normal, appearing as if there is a great vaso-constriction throughout the kidney.

The glomeruli that stop as a result of this stimulation generally show some degree of stasis during their stoppage; a lesser number show capillaries fairly bulging with red cells, and others show a gradual emptying of the capillaries with no inflow of red cells so that the glomerulus shows itself finally perfectly empty of cells. In most cases where the afferent vessel was visible, this also showed a stoppage of flow during the period of stimulation and a gradual emptying of cells into the glomerulus, after which the afferent artery could no longer be seen. This would indicate a point of

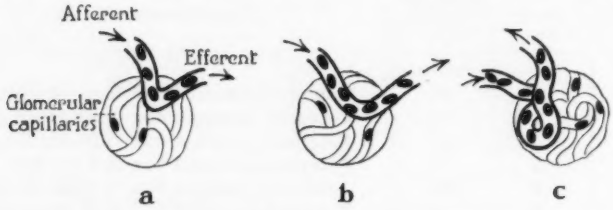

Fig. 1. Diagram of glomeruli from the frog's kidney showing the "shunt" between afferent and efferent glomerular arterioles.
Afferent = afferent glomerular arteriole.
Efferent = efferent glomerular arteriole.

great constriction away from the glomerulus, probably at the point of its origin, as has been shown by Krogh (1922). Then when the electrical stimulation was discontinued, the afferent artery would suddenly show a burst of flow, following which the entire glomerulus would burst into activity, or in other cases, the capillaries would assume their flow, one by one, over a period of five to ten seconds.

The glomeruli which do not stop as a result of splanchnic stimulation, sometimes appear to show a flow at an increased speed, as if a certain amount of arterial blood must get into the kidney regardless of the number of glomeruli that are open for flow.

Another interesting point in glomerular blood flow was observed as a result of peripheral splanchnic stimulation. In some glomeruli where this stimulation had decreased the glomerular capillary flow, one and occasionally two capillaries remained which, with the afferent and efferent arteries, showed a very rapid flow. When this was observed it was found prac-

tically impossible to stop the flow with ordinary electrical stimulation. In every kidney studied in this portion of the work, one or two, and as high as ten of these "shunt" arrangements have been observed. In most cases the connecting link appeared short, but in a few it was relatively large; for example, *c* in figure 1. This figure also shows several other glomeruli with a short connecting link.

Else and Dehoff (1919), in mammalian kidneys injected with dyes, have observed that the afferent and efferent arterioles in some cases were injected, whereas the glomerular capillaries have remained entirely free of the dye. They concluded that some sort of a direct loop between these two arterioles within the glomerulus must exist to explain their finding. This same loop must exist in the frog's kidney, but just how common it is, it is

TABLE 3

Effect of electrical stimulation of central end of sensory nerves

FROG NUMBER	BEFORE STIMULATION		SECONDARY COIL SET AT	DURATION OF STIMULATION	END OF STIMULATION		FIVE MINUTES LATER	
	Act.	Inact.			Act.	Inact.	Act.	Inact.
Central end of cut left sciatic								
			cm.	*minutes*				
1	34	6	12	4	6	21	26	16
2	45	10	6	3	15	31	38	12
3	36	8	12	5	24	15	32	6
Central end of cut left vagus								
4	58	6	12	4	26	20	42	12
5	30	10	12	4	12	32	20	16
6	30	15	12	6	20	20	31	13

Act. = glomeruli showing blood flow. Inact. = glomeruli showing no blood flow.

difficult to say, for in the case of many glomeruli the afferent and efferent vessels are entirely hid from view. It must be added that these connecting loops have so far been seen only in the kidneys of Rana catesbiana. No attempt so far has been made to look for them in Rana pipiens, because the splanchnic trunks are much smaller and are consequently much more difficult to manipulate without injury.

Stimulation of the central ends of cut sensory nerves such as the sciatic and vagus also produces this picture. In table 3 can be seen the effects produced on the number of active and inactive glomeruli again in the kidneys of Rana catesbiana. In both cases the number of active glomeruli are cut roughly in half. Frog 1 shows almost an eighty per cent decrease in the number of active glomeruli—a greater decrease than was obtained in splanchnic stimulation. Possibly this is due to the fact that in the frog

there are three fairly large sympathetic branches and sometimes a few very small fibers given off which run towards the aorta. For a complete effect, therefore, from splanchnic stimulation, all of these branches should be included, but up to the present this has been found impossible of completion. When sensory stimulation is discontinued, the glomeruli tend to return to normal as regards active flow. It can be seen from table 3 that five minutes after the stimulation is discontinued, the glomerular flow is fairly close to the previous level. In small fields of active glomeruli where

TABLE 4

Effect of electric sensory stimulation on small fields of glomeruli

EXPERIMENT NUMBER	ACTIVE	SECONDARY COIL SET AT 10 CM.	RESULT OF STIMULATION
			Central end of cut left sciatic
		seconds	
1	5	10	4 stopped, resumed flow in 2 minutes
2	2	10	2 stopped, resumed flow in 1 minute
3	3	15	3 stopped, resumed flow in 3½ minutes
4	2	5	2 stopped, resumed flow in 2 minutes
5	2	5	2 stopped, resumed flow in ½–1 minute
6	5	10	5 stopped, 3 resumed flow in 30 seconds
			2 resumed flow in 2 minutes
7	4	5	3 stopped, resumed flow in 40 seconds
8	5	60	3 stopped, resumed flow in 2 minutes
			Central end of cut left vagus
1	3	10	3 stopped, resumed flow in 2 minutes
2	5	10	5 stopped, 3 resumed flow in 20 seconds
			2 resumed flow in 3 minutes
3	2	15	2 stopped, resumed flow in 30 seconds
4	3	10	3 stopped, resumed flow in 1–3 minutes
5	5	5	2 stopped, resumed flow in 2 minutes
6	3	30	2 stopped, resumed flow in 2 minutes

Active = glomeruli showing blood flow.

the effect of stimulation could be observed continuously, the pictures produced are similar to those obtained with peripheral splanchnic stimulation. Table 4 shows these effects of stimulation of the central ends of the cut sciatic and vagus. An occasional "shunt" arrangement between the afferent and efferent arterioles within the glomerulus has also been noted here.

If stimulation of the splanchnics leads to decreased glomerular flow, one naturally wonders what section of the splanchnics will produce. This has been determined upon a series of Rana pipien and Rana catesbiana, and the results are shown in table 5. In every kidney studied, and especially in

those kidneys showing a large proportion of inactive glomeruli, section of all the visible trunks leading from the sympathetic chain toward the aorta, on one side, has resulted in an increase of active glomeruli on the same side. Out of thirteen experiments, in eight this has been controlled by counts of the glomeruli in the opposite or normal kidney During the time of the experiment, which has run from one to two hours, no marked change in these results have been observed. Richards and Schmidt (1924) mention that after destruction of the brain and spinal cord, intermittency of glomerular blood flow "may" still be observed. They report only upon one experiment in which they report their findings only for one small field of

TABLE 5

Effect of section of left splanchnic nerves upon active glomeruli

FROG NUMBER	LEFT KIDNEY				RIGHT KIDNEY			
	Before		After		Before		After	
	Active	Inactive	Active	Inactive	Active	Inactive	Active	Inactive
1	36	24	80	0				
2	12	25	53	0				
3	24	14	53	0	19	22	23	16
4	26	5	45	3				
5	57	4	86	1	60	4	65	2
6	75	6	86	1	75	9	68	4
7	77	4	93	0	73	3	70	7
8	115	2	127	0	120	5	109	11
9	24	15	60	0	15	12	30	13
10	74	4	96	0	72	2	74	5
11	94	4	104	0	95	2	103	2
12	72	1	89	0				
13	22	10	74	0				

Active = glomeruli showing blood flow. Inactive = glomeruli showing no blood flow.

glomeruli. In the experiments here reported, and in those following in this paper, where the splanchnics were sectioned this has been the exception rather than the rule. The number of active glomeruli after splanchnic section has remained extremely constant up to one and one-half hours following the section. Occasionally, one glomerulus out of seventy to one hundred may show arrest of flow, but when this has occurred, the periods of intermittency generally have been long (5 to 10 minutes). During the arrest the red cells in the glomerulus were very apt to show the purplish color of cyanosis. In many cases the glomeruli which showed arrest of flow were apt to continue this way throughout the time of the experiment. In the experiments where both kidneys were observed, the normal kidneys

showed good and frequent intermittencies of flow, whereas the kidney minus splanchnics showed none.

In two frogs (Rana catesbiana) where observations were carried on for over three hours, several small fields in a kidney in each animal were found where the glomeruli showed intermittency of blood flow after splanchnic section. In one field were three active glomeruli which during a period of five minutes showed flow for periods of from 10 to 25 seconds, then arrest of flow for 10 to 20 seconds. During thirty minutes these three glomeruli showed at various times a similar picture. In the case of two of these glomeruli the afferent artery could be seen for a distance of about four times the diameter of the glomerulus before it entered Bowman's capsule. During the periods of arrest these afferent arterioles also showed arrest of flow and a complete emptying of red cells into the glomerular capillaries. In another field one glomerulus was observed that showed an intermittent flow. In this case the flow and rest periods each lasted about thirty seconds. The results described for these two fields occurred three hours after the splanchnics were cut. They were not observed before this. In the other frog, again three hours after the splanchnics were cut, five glomeruli were found that showed periods of arrest lasting up to thirty seconds, alternating between periods of flow which lasted considerably longer, generally two to three minutes.

These findings may be due to several factors: 1, this intermittent flow may be a finding associa ed with a gradual fall in blood pressure and decrease of good respiratory movements; 2, it may be due to an inherent property of the afferent arteriole to contract more or less rhythmically. This is perhaps analogous to the revival of tonus in arterioles and capillaries in muscles as has been shown by Krogh (1922). The denervated ureter, both inside of the body as in the kidney transplanation studies of Capelle (1908) and Ibuka (1926), and outside of the body (Roth, 1916), will show contractions which in the cases of denervated kidneys will force a spurt of urine for a distance of twelve inches. Also the "Musculus Spiralis Papillae" of Muschat (1928), when removed from the kidney pelvis, beat rhythmically for an hour. But in all of these experiments there was a period of inhibition before the rhythmic contractions began. This latter explanation seems to be the more logical one.

The discrepancy between the observations of Richards and Schmidt (1924), and those reported here, may perhaps be reconciled by the findings of Ellinger and Hirt (1925) who reported that section of the spinal roots in mammals did not produce as marked a diuresis as section of the splanchnics; therefore, the spinal ganglia must exert some force through the sympathetic fibers in the splanchnics.

Finally, most observers have found that kidneys with sectioned splanchnics at first put out urine in amounts increased over the normal, but that

TABLE 6

Reflex arrest of glomerular flow

Pinching ureter

FEOG NUMBER		TIME	ACTIVE	INACTIVE
1		3:15	118	5
	Clamp on left ureter	3:16		
		3:18	89	3*
		3:30	118	4
2		4:35	48	7
	Clamp on left ureter	4:37		
		4:38	23	12*
		4:40	30	7
		4:45	32	3
		4:55	44	5
3		5:15	67	45
	Clamp on left ureter	5:17		
		5:18	34	79*
		5:30	69	42
4		11:35	90	1
	Clamp on left ureter	11:40		
		11:42	61	7*
		11:50	87	2
5		4:30	78	5
	Clamp on left ureter	4:32		
		4:35	46	12*
		4:40	81	5
6		3:15	76	2
	Clamp on left ureter	3:17		
		3:20	48	10*
		3:25	84	4
7		4:50	30	10
	Clamp on right ureter	4:52		
		4:55	8	8*
		5:00	26	8
		5:15	31	8
8		2:45	102	3
	Clamp on right ureter	2:47		
		2:50	85	7*
		3:00	96	5
		3:10	100	5

Active = glomeruli showing blood flow. Inactive = glomeruli showing no blood flow.

* Clamp removed (after second recorded count).

eventually the urine volume tends to return to normal. If the observations reported here, that at some time after splanchnic section the glomeruli tend to show some intermittency of flow, are correct, these two types of observations may now be joined. The immediate effects of splanchnic section are to increase the number of active glomeruli and to increase the volume of urine secreted, but that sooner or later the afferent arteries and

TABLE 7

Effect of section of left splanchnic on reflex arrest of glomerular flow

FROG NUMBER		TIME	LEFT KIDNEY	
			Active	Inactive
1		4:10	71	0
	Clamp on left ureter	4:13		
		4:15	71	0*
		4:20	72	0
2		10:30	65	0
	Clamp on left ureter	10:32		
		10:35	65	0*
		10:40	66	0
3		4:45	71	0
	Clamp on left ureter	4:47		
		4:50	73	0*
		4:55	72	0
4		3:45	104	2
	Clamp on left ureter	3:47		
		3:50	104	2*
		3:55	103	3
5		11:30	84	0
	Clamp on left ureter	11:32		
		11:35	84	0*
		11:40	83	0

Active = glomeruli showing blood flow. Inactive = glomeruli showing no blood flow.

* Clamp removed.

glomeruli establish their own intermittency without nerve control and then the urine volume tends to approximate the orginal normal level.

Reflex anuria is a condition met with both experimentally and clinically. This condition generally arises from some point of excessive stimulation. It has been reported by Loucks and Scott (1928) from pulling on the kidney, stretching muscles and incising the skin in dogs. It has been reported by clinicians from inserting a ureteral catheter into the ureter and from

TABLE 8

Effect of cocaine on reflex arrest of glomerular flow

FROG NUMBER		TIME	LEFT KIDNEY	
			Active	Inactive
1		4:30	78	5
	Clamp on left ureter	4:32		
		4:35	46	12*
		4:40	81	5
	Cocaine HCl 1 per cent run into ureter	4:45		
		4:50	84	3
	Clamp on left ureter	4:52		
		4:55	83	3*
		5:00	85	2
2		10:30	66	6
	Clamp on left ureter	10:32		
		10:35	32	14*
		10:45	65	8
	Cocaine HCl 1 per cent run into ureter	10:47		
		10:55	63	9
	Clamp on left ureter	10:57		
		11:00	65	5*
		11:05	67	4
3		1:45	70	2
	Clamp on left ureter	1:48		
		1:50	47	13*
		2:00	71	2
	Cocaine HCl 1 per cent run into ureter	2:03		
		2:05	51	14
		2:10	65	2
	Clamp on left ureter	2:12		
		2:15	65	6*
4		4:10	73	6
	Clamp on left ureter	4:14		
		4:15	56	11*
		4:20	78	8
	Cocaine HCl 1 per cent locally to left ureter	4:25		
		4:30	80	3
	Clamp on left ureter	4:33		
		4:35	82	0*
	$HgCl_2$ 2 per cent locally to left ureter	4:40		
		4:43	82	2

Active = glomeruli showing blood flow. Inactive = glomeruli showing no blood flow.

* Clamp removed.

instilling drugs such as silver nitrate into the ureter, and its association with kidney stones and ureteral kinks is also well known. Blum (1907) reported a case of complete anuria resulting from a surgical procedure in which the ureter was probably caught in a ligature. Because of its importance, therefore, and because of what we know of the control of the splanchnics on kidney blood flow and urine output, it was thought worth while to try

TABLE 9

Reflex arrest of glomerular flow

Irritants applied to ureter externally

FROG NUMBER		TIME	ACTIVE	INACTIVE
1		11:55	88	2
	$AgNO_3$ 10 per cent to ureter	11:57		
		11:59	70	8
		12:10	81	7
		12:15	82	7
2		4:45	75	3
	$HgCl_2$ 2 per cent to ureter	4:48		
		4:50	56	5
		4:55	70	2
		5:00	73	5
3		4:15	73	5
		4:25	74	3
	$HgCl_2$ 2 per cent to ureter	4:27		
		4:30	28	34
		4:35	51	18
		4:40	62	9
		4:45	64	9
4		3:25	64	8
	$AgNO_3$ 10 per cent to ureter	3:27		
		3:30	36	15
		3:35	42	10
		3:45	64	4

Active = glomeruli showing blood flow. Inactive = glomeruli showing no blood flow.

the effect of some of the procedures known to produce anuria upon the activity of the glomeruli, both with normal innervation and without splanchnic innervation.

From the reflex point of view, attention should be called to the findings from this laboratory which go back six or seven years, that immediately after a frog is prepared for microscopic observations, as is described by Bieter and Hirschfelder (1924), there are not as many active glomeruli as can

be seen 10 to 20 minutes later. In the light of the following work, this is probably the result of reflex closure of the afferent glomerular arteriole.

The effect of placing a small bull-dog clamp on the ureter was first tried. Table 6 shows the results. In every case the number of active glomeruli dropped, giving an average decrease for all of the experiments of about forty per cent. The clamp was removed immediately after this count, following which the number of active glomeruli began to increase. During the next ten or fifteen minutes the glomeruli again reached normal levels.

TABLE 10

Section of splanchnics on reflex arrest of glomerular flow

FROG NUMBER		TIME	ACTIVE	INACTIVE
1		8:00	79	0
	$HgCl_2$ 2 per cent to ureter externally	8:02		
		8:05	79	0
		8:10	80	0
2		4:55	64	0
	$HgCl_2$ 2 per cent to ureter externally	4:57		
		5:00	64	0
		5:10	65	0
3		3:10	68	0
	$AgNO_3$ 10 per cent to ureter externally	3:12		
		3:15	68	0
		3:20	68	0
4		11:30	45	0
	$AgNO_3$ 10 per cent to ureter externally	11:32		
		11:35	45	0
		11:40	46	1

The splanchnic nerves on the side studied were sectioned before the irritants were applied to the ureters.

On the other hand, when the splanchnic nerves were cut, this decrease in active glomeruli was not observed. From table 7 it can be seen that the number of active glomeruli showed no change at all. Further, it was noticed that there was no appreciable change in rate of flow or size of glomeruli. In this connection, it is interesting to recall that Quinby (1916) mentioned that in handling the ureters of denervated kidneys, the temporary inhibition of urine flow so often seen in normal kidneys after handling the ureter, was not observed, and that he thought this normal inhibition of urine flow was analogous to that occasionally seen clinically upon the passage of a ureteral catheter.

The effect of 1 per cent cocaine hydrochloride upon this reflex arrest of

glomerular flow by pinching the ureter was also tried. Table 8 shows the result of placing a small bull-dog clamp in the normal ureter with the resulting decrease in active glomeruli. In each animal this is followed by running 1 per cent cocaine into the ureter at a pressure of 4 to 5 cm. water. Following this, the clamp was again applied, but this time there was no effect upon the number of active glomeruli nor upon the character of individual flow.

TABLE 11

Reflex arrest of glomerular flow

Part A—Edema fluid on glomeruli with intact splanchnic innervation

FROG NUMBER	BEFORE		AFTER LIGATURE REMOVED, LEG MASSAGED TWO MINUTES					
			Immediate		Ten Min. Later		Twenty Min. Later	
	Act.	Inact.	Act.	Inact.	Act.	Inact.	Act.	Inact.
1	56	3	38	7	26	12	35	12
2	86	11	70	13	77	12	79	17
3	52	2	29	15	38	6	50	5
4	45	7	16	23	22	21	27	14
5	74	23	44	16	59	16	75	12

Part B—Edema fluid on glomeruli with and without splanchnic innervation

FROG NUMBER	LEFT KIDNEY—SPLANCHNICS CUT						RIGHT KIDNEY—INTACT SPLANCHNICS					
	Before		After ligature removed leg massaged two minutes				Before		After ligature removed leg massaged two minutes			
			Immediate		One hour later				Immediate		One hour later	
	Act.	Inact.	Act.	Inact.	Act.	Inact.	Act.	Inact.	Act.	Inact.	Act.	Inact.
1	90	1	86	1	88	0	68	4	27	14	60	4
2	93	0	93	0	93	0	70	7	12	33	59	4
3	126	0	126	0	128	0	117	13	48	43	120	4
4	96	0	98	0	96	0	72	5	47	13	70	10
5	98	2	96	2	103	3	103	5	26	25	102	2
6	60	0	65	0	64	0	28	14	13	11	24	6

Act. = glomeruli showing blood flow. Inact. = glomeruli showing no blood flow.

Irritants such as silver nitrate 10 per cent and mercury bichloride 2 per cent, applied to the ureter externally, also show the characteristic arrest of glomerular flow. This is illustrated in table 9. Again this decrease of glomerular flow is absent when the splanchnics are cut, as is shown in table 10.

Andrews (1921), while working upon experimental uremia, noticed that when the legs of dogs developed an edema from too tight a ligature, if then

the ligature was removed and the leg massaged, he obtained an anuria. Because of this finding, rubber binders were placed on the legs of a series of frogs, and in 12 to 15 hours a marked edema was produced. The leg below the ligature was from one-half to again as large as the normal leg. The skin was slightly opaque and in half the cases the skin was slightly injected, as compared with the normal leg. At the end of the above interval, the frog was prepared for microscopic observation and when the normal counts of glomeruli were obtained, the ligature was removed and the leg massaged very gently. As can be seen in table 11, part A, this resulted in a marked decrease of active glomeruli and the return to normal was not complete for 15 to 20 minutes. In table 11, part B, can be seen the effects of the same type of edema fluid on the glomeruli in the left kidney with the splanchnics cut, and on the glomeruli in the right kidney with intact nerves. Here again the effect was removed by section of the splanchnics. In the normal kidney, the active glomeruli were cut down by almost fifty per cent, whereas in the kidney without splanchnic innervation, no effect upon the number of active glomeruli was produced. Some slowing of the rate of flow was noticed in two of the kidneys minus splanchnics. It was thought that this might have been due to a lowering of blood pressure. In the second animal showing a slowing of blood flow in the kidney minus splanchnics from the edema fluid, blood pressure readings were taken which showed the following:

Before ligature was removed	$\frac{32}{20}$ mm. Hg
Immediately after ligature was removed	$\frac{30}{20}$ mm. Hg
Five minutes later	$\frac{29}{20}$ mm. Hg
Fifteen minutes later	$\frac{29}{18}$ mm. Hg

From these figures it can be seen that there is no marked lowering of blood pressure, yet this slowing of blood flow was apparent for at least five minutes. The results of these edema experiments on glomerular blood flow indicate again that the splanchnics play a major rôle in their arrest.

In table 12 are shown the results of similar types of massage, as was used on the edematous legs, on normal legs. Here no definite effect on the number of active glomeruli was produced.

Lastly, the reflex effects on glomerular blood flow from irritants such as bichloride of mercury solution injected into the ureter and into the tubules was determined.

When mercuric chloride up to 1 per cent solution was injected into the ureter and collecting ducts by means of a cannula, at a pressure of 10 cm. of water, for from 30 to 60 seconds, a marked decrease of glomerular blood

flow was obtained. By means of deeply colored dye solutions it has been ascertained that at a pressure of 5 to 10 cm. of water for one minute, the ureter and some of the larger collecting ducts are injected, whereas at a pressure of 25 to 30 cm. of water for one minute, the distal and most of the proximal convoluted tubules are injected. Rarely at these pressures does colored fluid enter the capsule of Bowman. This decrease in glomerular blood flow again amounts to about fifty per cent of the glomeruli showing active flow, and the effect lasts up to 30 to 50 minutes. The changes in the number of active glomeruli are shown in table 13, part A. In part B of this table is shown the effect of injecting cocaine HCl 1 per cent into the

TABLE 12

Effect of massage on normal leg

FROG NUMBER		TIME	LEFT KIDNEY		RIGHT KIDNEY	
			Act.	Inact.	Act.	Inact.
1		11:05	78	4	87	2
	Massage 2 minutes right leg	11:12				
		11:15	76	3	88	6
		11:25	79	8	81	7
	Massage 2 minutes right leg	11:32				
		11:35	90	0	95	2
2		4:05	65	8	46	6
	Massage 2 minutes right leg	4:07				
		4:10	73	6	51	8
3		2:00	70	4	73	2
	Massage 2 minutes right leg	2:02				
		2:05	68	8	71	2

Active = glomeruli showing blood flow. Inactive = glomeruli showing no blood flow.

ureter before the mercury solution. Here also is shown an initial decrease in the number of active glomeruli from the injection of cocaine, which, however, disappears within five minutes. Following this cocainization which is carried out in a manner similar to the other injections at a pressure of 10 cm. of water, the bichloride of mercury solutions were again injected, but in these cases without any reflex arrest of glomerular flow.

An attempt was now made to anesthetize the ureter and larger collecting ducts as above, after which bichloride of mercury solution was injected into the ureteral cannula at a pressure of 25 cm. of water, with the idea of determining the presence of sensory nerves within the tubules or between the epithelial cells. In two frogs so cocainized, mercuric chloride 0.1 per

cent and 0.01 per cent produced a decrease in active glomeruli from 41 and 74 per field, to 31 and 61 per field, respectively. This effect was not as marked as that obtained from the ureters and collecting ducts alone. Because of the possibility of cocaine being absorbed and to a certain extent

TABLE 13

Reflex arrest of glomerular flow from injections into ureter

FROG NUMBER	NORMAL		INJECTION INTO URETER AND COLLECTING DUCTS	IMMEDIATE EFFECTS		TEN MINUTES LATER		THIRTY MINUTES LATER		FIFTY MINUTES LATER	
	Act.	Inact.		Act.	Inact.	Act.	Inact.	Act.	Inact.	Act.	Inact.
(a) Normal glomerular effects											
1	81	3	0.01 per cent $HgCl_2$ 5 cm. H_2O pressure for 1 minute	23	21	32	13	58	13	68	14
2	72	5	0.0001 per cent $HgCl_2$ 10 cm. H_2O pressure for 1 minute	45	19	67	8	70	8		
3	43	3	0.0001 per cent $HgCl_2$ 10 cm. H_2O pressure for 1 minute	25	12	49	6				
4	38	12	0.1 per cent $HgCl_2$ 10 cm. H_2O pressure for 40 seconds	13	27	27	13	44	6		
5	98	3	0.01 per cent $HgCl_2$ 10 cm. H_2O pressure for 1 minute	66	16	75	10	86	6		
(b) After cocaine HCl 1 per cent into ureter at 10 cm. H_2O pressure											
1	41	8	Cocaine injected	8	34	41	8				
	42	8	0.001 per cent $HgCl_2$ 10 cm. H_2O pressure for 1 minute	40	8	42	8	42	6		
2	72	2	Cocaine injected	40	15	73	1				
	73	1	0.01 per cent $HgCl_2$ 10 cm. H_2O pressure for 1 minute	73	0	73	2	70	2		
3	65	8	Cocaine injected	35	20	66	7				
	66	7	0.1 per cent $HgCl_2$ 10 cm. H_2O pressure for 1 minute	65	8	64	6	68	5		
4	81	5	Cocaine injected	71	6	84	3				
	84	3	0.1 per cent $HgCl_2$ 10 cm. H_2O pressure for 1 minute	84	2	84	2	84	2		

Act. = glomeruli showing flow of blood. Inact. = glomeruli showing no flow of blood.

being distributed throughout the kidney by diffusion, this type of experiment was discontinued.

In the series of frogs shown in table 14, mercuric chloride was injected through ureteral cannula at a pressure of 25 cm. of water. This procedure again resulted in arrest of glomerular flow, the average decrease for all of the experiments being a little less than fifty per cent. It can thus be seen

that this closure of the glomerular capillaries to blood flow is produced to no greater degree when the irritant mercury salt solution is run into the tubules as well as the ureter and collecting ducts.

This appears, to a certain extent, to be physiological confirmation of the anatomical work of Smirnow (1901). This investigator, studying the kidneys of mammals including man, and frogs, found nerve fibers running in between the epithelial cells of the tubules, collecting duct, and also ureter. Those ending in the tubules, because of their similarity to nerve endings in secreting glands in the body, he concluded to be secretory; whereas those

TABLE 14

Reflex arrest of glomerular flow

Effect of injections into ureter and tubules

FROG NUMBER	NORMAL		INJECTION OF DRUG	IMMEDIATE EFFECTS		TEN MINUTES LATER		THIRTY MINUTES LATER		FIFTY MINUTES LATER	
	Act.	Inact.		Act.	Inact.	Act.	Inact.	Act.	Inact.	Act.	Inact.
1	30	8	0.1 per cent $HgCl_2$ 25 cm. H_2O pressure for ½ minute	12	20	18	12	20	12	27	10
2	76	1	0.1 per cent $HgCl_2$ 25 cm. H_2O pressure for 1 minute	50	13	62	10	60	6	68	4
3	65	2	1 per cent $HgCl_2$ 25 cm. H_2O pressure for 1 minute	34	15	40	8	58	3		
4	57	2	0.5 per cent $HgCl_2$ 25 cm. H_2O pressure for 1 minute	28	12	36	12	55	5	55	3
5	73	4	0.5 per cent $HgCl_2$ 25 cm. H_2O pressure for 1 minute	45	19	50	15	60	9	69	7
6	32	7	0.5 per cent $HgCl_2$ 25 cm. H_2O pressure for 1 minute	17	19	10	23	26	15	24	16

Active = glomeruli showing blood flow. Inactive = glomeruli showing no blood flow.

ending farther down in the urinary system, in the collecting ducts, pelvis and ureter, being more simple in structure than the above, he concuded to be sensory. In the experiments herein reported, a more powerful arrest of glomerular flow was obtained by irritating the ureter and collecting ducts; at least this effect was just as strong as that produced from irritation of the ureter, collecting ducts and tubules. This tends to indicate the presence of more sensory nerve endings in the ureter and collecting ducts than in the tubules proper of the kidney. However, much more work is necessary before this point can be satisfactorily settled.

When the splanchnics in a series of frogs were again sectioned (table 15), the injections of solutions into the ureter and tubules of the kidney were again without effect on the glomerular blood flow. The arrest of the flow of

blood through the glomerular capillaries obtained by irritation within the ureter, collecting ducts and tubules, like that produced by the other measures employed, is in all probability a reflex vaso-constriction, for which the sympathetic fibers running to the kidney are essential.

The findings here reported upon the function of the splanchnics when stimulated directly or reflexly to decrease glomerular blood flow and in all

TABLE 15

Effect of section of splanchnics on reflex arrest of glomerular flow

FROG NUMBER	NORMAL		INJECTION OF DRUG	IMMEDIATE EFFECTS		TEN MINUTES LATER		THIRTY MINUTES LATER	
	Act.	Inact.		Act.	Inact.	Act.	Inact.	Act.	Inact.
(a) Injections into ureter									
1	74	0	2 per cent $HgCl_2$ 10 cm. H_2O pressure for 1 minute	73	0	74	0	74	0
2	64	0	0.01 per cent $HgCl_2$ 10 cm. H_2O pressure for 1 minute	65	1	65	1	65	1
3	73	0	0.5 per cent $HgCl_2$ 10 cm. H_2O pressure for 1 minute	72	1	72	1	72	1
4	68	3	0.1 per cent $HgCl_2$ 10 cm. H_2O pressure for 1 minute	68	3	68	3	67	3
(b) Injections into tubules									
5	62	2	1 per cent $HgCl_2$ 25 cm. H_2O pressure for 1 minute	62	2	62	2	63	2
6	98	0	0.5 per cent $HgCl_2$ 25 cm. H_2O pressure for 1 minute	102	0	102	0		
7	25	1	0.5 per cent $HgCl_2$ 25 cm. H_2O pressure for 1 minute	25	1	25	1		
8	49	3	0.1 per cent $HgCl_2$ 25 cm. H_2O pressure for 1 minute	49	3	49	3		

The splanchnic nerves on the side studied were sectioned before the ureteral injections were made.

probability glomerular filtration, and the inhibition of this function by local anesthetization of the part stimulated, to produce this reflex, such as the ureter, and by section of the splanchnics, is thought to have a clinical bearing. Whereas these results were obtained in the frog, it is likely that in the frog the vaso-motor control of the kidney vessels is not as highly developed as it is in mammals and man, and consequently, ascending the scale, the effects would possibly be more powerful. In so far as they are thus able, these results are thought to add confirmations: 1 to the report of Neuwirt (1923) who with procaine anesthesia of the splanchnics in a case

of kidney stone colic, relieved the pain and produced a resumption of urine secretion; 2, to the reports of Hess (1926), (1928) who performed renal sympathectomy in five cases, the result of which was an increased output of urine from the operated kidney, and 3, to the report of Rowntree (1928) who has studied renal sympathectomy in dogs with similar findings, preparatory to applying the procedure clinically.

CONCLUSIONS

1. Intermittency of glomerular blood flow and intermittency of capillary flow within the glomerulus in the normal frog's kidney, as first reported by Richards (1922), is again confirmed.

2. Immediately after splanchnic section in the frog, this intermittency, for the most part, disappears. At three hours after splanchnic section in two cases this intermittency was again to be seen in several small fields of glomeruli, but the rest periods were no longer than thirty seconds.

3. Section of the splanchnics, as an acute effect, results in an increase of glomeruli showing active blood flow.

4. Various measures such as pinching the ureter, applying an irritant locally, both externally and internally, and edema fluid let into the general circulation, result in reflex arrest of glomerular flow.

5. This reflex arrest of glomerular flow is prevented by splanchnic section and cocainization of the part stimulated.

6. The reflex arrest produced by mercuric chloride in the ureter and collecting ducts appears more pronounced than the reflex arrest coming from within the kidney tubules.

7. These experiments show the marked effect of the splanchnics upon glomerular blood flow and, as far as they are able, tend to explain how local anesthesia and section of renal nerves will produce beneficial results clinically in reflex oliguria and anuria.

I wish to express my sincere appreciation to Dr. A. D. Hirschfelder, Director of the Department of Pharmacology, in whose department the above experiments were carried out; also to Dr. F. H. Scott, Department of Physiology, Dr. George E. Fahr, Department of Medicine, and Dr. Leonard G. Rowntree of the Mayo Foundation, Rochester, for their helpful advice and criticism.

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THE ACTION OF AMYTAL IN IMPAIRING VAGUS CARDIAC INHIBITORY EFFECTS, AND OF ETHER IN INCREASING THE RESPIRATORY RATE AFTER ITS DEPRESSION BY AMYTAL

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During a class demonstration experiment in which amytal was used as the anesthetic on a dog certain unusual responses were obtained upon stimulation of the intact vagus nerves. The heart was slowed very little upon faradic stimulation of the vagi, and instead of a fall in blood pressure an immediate marked rise in pressure was recorded. Gaynor and Underwood became interested and, in an attempt to determine the cause of the apparent irregularities, they and the senior author carried out experiments with amytal on seventeen dogs (fifteen of which were under observation for four or more hours before death) in the spring of 1929. In preparing the amytal for use on the dogs, a weighed amount of dry amytal powder was stirred into a little water and then brought into solution by neutralizing with 20 per cent NaOH solution. The NaOH was added cautiously drop by drop with constant stirring until only one or two crystals of amytal remained undissolved. Water was then added to bring the amytal to 10 per cent. This solution of the sodium salt of amytal as thus obtained was administered intraperitoneally—the dose given being of such volume as to use 55 mgm. of the dry amytal powder per kilo of body weight. This is about half the amount of the fatal dose as reported by Eddy (1928), and it was usually sufficient to produce complete and satisfactory anesthesia. In case of some dogs smaller additional doses were administered in the same manner as the first dose.

Our experiments fall into three groups, viz.: 1, those in which amytal alone was given; 2, those in which ether was first used, followed by decerebration (with discontinuance of ether) and then, after the effects of the ether had passed, by amytal; and 3, those in which small varying amounts of ether were used after the animal was first completely anesthetized by amytal.

When we began our work we were acquainted with the published findings that amytal first increased heart rate and then slowed it, and that the respiratory rate was slowed by amytal (Eddy, l.c., and others). We were not

acquainted with work describing an effect of any barbital compounds upon the vagus nerve inhibitory mechanism in the heart. Later, just as our experiments were finished, Dr. M. L. Tainter kindly brought to our attention a paper (De Waele, 1925) in which it was reported that the barbital compound, "Somnifene," in strong doses affected the peripheral part of the inhibitory mechanism of the heart showing excitation at the very first and paralysis afterward. At the proper moment of this paralysis, according to the report, the vagus "almost does not respond to electric excitation." This latter finding was similar to the effect we had obtained in our experiments with amytal. Then, also, was published a paper (Lieb and Mulinos, May 1929) stating that solutions of sodium amytal injected intravenously 1, depressed the circulation, and 2, caused prolonged but temporary paralysis of the vagus to the heart. The latter conclusion was reached after the authors had observed the effect of electrical stimuli on the peripheral ends of the cut vagi at different stages of amytal anesthesia and had compared results so obtained with each other, and with the effect of vagus stimulation under ether anesthesia. Shortly after amytal was administered, such stimulation practically failed to inhibit the heart but two hours later similar stimuli caused marked slowing of the heart comparable to that obtained under ether anesthesia.

This paper of Lieb and Mulinos renders unnecessary a detailed report of our first group of experiments. In general our findings confirm the two results of Lieb and Mulinos stated above. We found that amytal impaired the effect of vagus stimulation on the heart and that this impairment of effect could not be overcome by prolonged faradic stimulation of maximal strength. We observed some recovery from this impairment but no case in which the *full* vagus inhibitory effect upon the heart was recovered under amytal anesthesia, although most of our animals were under observation for four hours and some for over five hours. The significance of this last statement will be brought out in the discussion of the experiments of group 2.

We obtained regularly a slight fall (of varying degree) in blood pressure upon administering small additional doses of amytal to dogs already anesthetized. Such animals, with low blood pressure, always showed congestion in the intestinal area.

Our experiments in groups 2 and 3 mentioned above enabled us to make certain further observations not indicated in the paper just cited. We may state briefly these observations and give typical graphic records. Our records for the group 2 experiments show that peripheral vagus stimulation (both vagi cut) with maximal faradic stimuli of 18 to 20 seconds' duration gave the greatest slowing of the heart in *decerebrate* dogs (even stopping the heart completely); it gave the least slowing of the heart in the same decerebrate dogs early in amytal anesthesia, and an intermediate

slowing in the same dogs under complete ether anesthesia before decerebration. Only two dogs in this group maintained a good blood pressure throughout the long period of the experiment, but they gave exactly similar results in this respect. In other words, ether, as well as amytal,

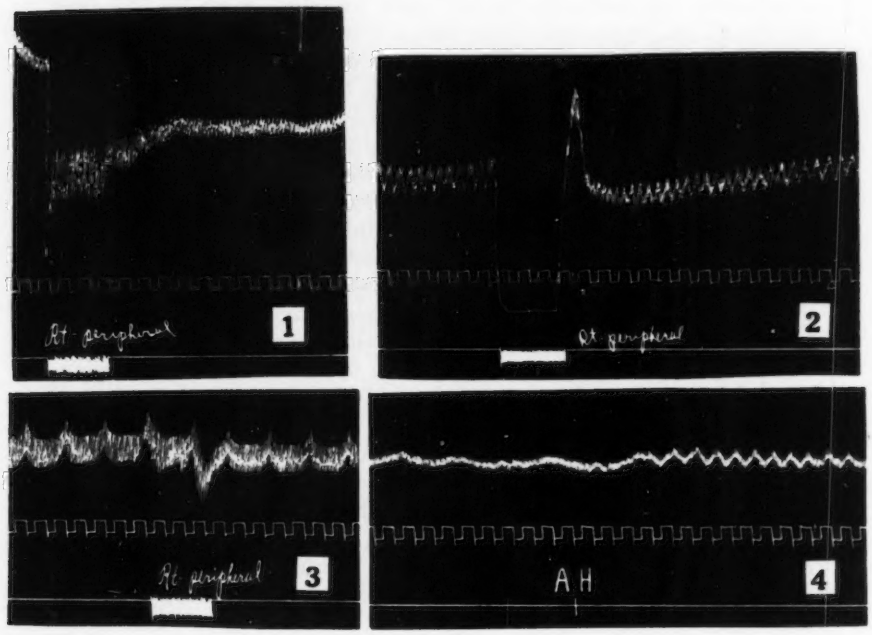

Fig. 1. Dog 11. Blood pressure record on stimulation of peripheral end of right vagus (both vagi cut), under *ether* anesthesia.

Fig. 2. Dog 11. Same stimulus, same vagus as in figure 1 but after *decerebration* and after dog was free from most of the ether. Heart entirely inhibited for about 20 seconds.

Fig. 3. Dog 11. Same stimulus, same vagus as in 1 and 2 but about 1 hour after *amytal* administration to the decerebrate animal. Note that respiratory rate is now depressed, somewhat, also.

Fig. 4. Dog 12, showing effect of a little ether, in the air breathed, on the respiratory rate (previously greatly slowed by amytal)—an almost immediate 5-fold increase, here. Blood pressure unchanged, although it often does fall a little.

impaired the effect of vagus stimulation upon the heart but its influence in this respect was not nearly so marked as that of the early effect of amytal. After two hours or so, when the vagus inhibitory mechanism had partly recovered from its impairment by amytal, stimulation of the peripheral end of the vagus slowed the heart *to about the same degree as was the*

case under ether anesthesia—not nearly so much as in the decerebrate animal, however.

Lieb and Mulinos evidently believed that stimulation of the vagus nerve of a dog under ether anesthesia gave the full, or normal, inhibitory effect of the vagus upon the heart. That belief naturally brought them to conclude that under amytal the vagus inhibitory fibers (at first *fully* paralyzed) had recovered entirely from amytal paralysis after 2.1 hours of anesthesia. They did not compare their results with the effect of vagus stimulation on the decerebrate dog. We have been unable to find a statement in the literature, hitherto, that ether impairs the effect of vagus inhibition upon the heart during complete ether anesthesia. The view evidently taken by Lieb and Mulinos would therefore seem to be the *usual* view held, namely, that maximal stimulation of the vagus nerve of a dog under complete ether anesthesia gives the full, normal inhibitory effect of the vagus upon the heart. Our experiments directly oppose this view. They show that the vagus of the decerebrate dog (without ether or any other anesthetic) is far more effective in inhibiting the heart than is the same nerve when the dog is under ether. Graphic records of the effect of vagus stimulation upon the heart of a dog under ether, under decerebration, and under amytal anesthesia, respectively, are given in figures 1, 2 and 3.

When a dog had just been placed under amytal anesthesia and the inhibitory effect of vagus stimulation upon the heart was almost absent, it was observed that sensory vagus effects upon the vasomotor centers were still present. This could always be seen when peripheral and central vagus effects were studied separately by electric stimulation, after cutting the vagi. The sensory effects might be pressor or depressor, of varying degree, depending upon the strength of the stimulus and the degree of anesthesia[1] —and varying somewhat, also, with the vagus nerve itself in different dogs —just as has already been found true for dogs under ether anesthesia. It seemed clear, then, why stimulation of the intact vagus nerve of an animal under amytal might give a marked rise instead of a fall in blood pressure as described at the beginning of this paper. By its strong paralytic action upon the peripheral vagus inhibitory mechanism in the heart, the amytal simply uncovered the effect of sensory vagus pressor impulses if such impulses happened to be present and dominant over depressor impulses in the nerve under stimulation—and a rise of blood pressure was the result. Stimulation of the same nerve when the animal was decerebrate (and without amytal), or when it was under ether anesthesia alone, would result in an abrupt fall in blood pressure because under both of the latter

[1] Strong stimuli were more apt to give pressor effects, and very weak stimuli depressor effects, but in deeper anesthesia even a strong stimulus that had formerly been pressor might become depressor in effect.

conditions the heart was either stopped or slowed so markedly as to swamp any vaso-constrictor effect as reflected in blood pressure.

Our experiments in group 3 show that, when the dogs were under amytal anesthesia so that the respiration rate had become decidedly slowed (i.e., as a result of amytal), administration of small amounts of ether with the air breathed caused almost an immediate increase in the rate of breathing. This effect of the ether was easy to obtain. We did not attempt to maintain the increased rate for very long periods but we observed that the margin of safety was not very great. If too much ether was given the respirations slowed again and then stopped altogether so that it was necessary to resort to artificial respiration in order to wash the ether vapor from the lungs and restore spontaneous respiratory movements again. This effect, with small amounts of ether, of quickening the respiratory rate after amytal depression could be obtained after both vagus nerves had been cut, so that it did not appear to be a reflex from the lungs. In order to determine whether possible irritating effects of the ether vapor upon the tracheal epithelium was responsible for sensory impulses which might be stimulating the respiratory center to more rapid discharges, we carried out the following test. A dog was placed under amytal as already described. The trachea was opened and the trachea cannula inserted as close as possible to the bronchi (i.e., without opening the chest). Then the trachea was cut entirely off next to the trachea-cannula. When the respiratory rate had become depressed below normal, now, both vagi were cut in the mid-neck region anterior to the branching of the recurrent laryngeal nerves. A cannula was then fitted into the free cut end of the trachea and air charged with ether vapor was puffed through the length of the trachea—and out at the mouth—for one minute, by means of a bellows. No noticeable change in respiratory rate resulted. The trial was repeated three times with no increase in the breathing rate. The animal was now allowed to breathe ether vapor in air through the trachea cannula and beginning with the second breath an increase in respiratory rate was noted. We believe that the possibiiity of this increase in rate of respiration being due to a reflex from sensory impulses over the vagus fibers from the lungs, or over the recurrent laryngeal, the superior laryngeal, or the ninth cranial nerves from the trachea and pharyngeal regions was thus ruled out. Figure 4 illustrates this effect of the ether. No further experiments designed to account for this influence of the ether upon the respiratory rate of an amytalized dog were tried by us.[2] The observation seems interesting when one remembers that ether likewise stimulates a

[2] Dr. V. E. Hall and Shafer have since obtained exactly the same effect (viz., marked increase in respiratory rate) by intravenous injection of small amounts of ether into a dog with breathing rate depressed under amytal. The effect was produced in each of three separate trials on the same animal.

subject whose respiratory movements are failing under nitrous oxide anesthesia—although the ether may possibly bring about the similar result in a different manner in the two cases.

CONCLUSIONS

1. Conclusions of Lieb and Mulinos that amytal rapidly impairs the effect of vagus stimulation upon the heart, and that it tends to depress the circulation are confirmed. After a time there is a gradual recovery from the impairment; however, we observed no case in which the vagus inhibitory mechanism of the heart recovered entirely while the dog was under amytal anesthesia.

2. Stimulation of the vagus gives the greatest slowing of the heart in dogs not under the influence of any anesthetic (but decerebrate); the least slowing in dogs just completely under amytal anesthesia; and an intermediate degree of slowing when the same animal is under ether anesthesia alone—or when it has been under amytal for about two hours or more.

3. Stimulation of the intact vagus nerve of a dog whose inhibitory mechanism in the heart is almost paralyzed with amytal may cause a marked rise in blood pressure (instead of a fall) if the nerve stimulated happens to carry strong pressor impulses.

4. Small amounts of ether given a dog either in the air breathed, or intravenously, quickly increases the breathing rate after it has been greatly depressed by amytal; and this effect of ether is shown not to be due to a reflex aroused by irritation of any part of the respiratory epithelium.

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THE ACTION OF INSULIN ON THE MOTILITY OF THE GASTRO-INTESTINAL TRACT

II. Action on the Heidenhain Pouch

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We have performed a series of experiments to determine under a variety of conditions the effect of insulin and of dextrose injections on the spontaneous motility (hunger contractions) of the stomach during fasting. These investigations have had two main objects: 1, to understand more thoroughly the mechanisms leading to the gastric stimulation of the normal empty stomach produced by insulin and the inhibition produced by dextrose, and 2, to obtain a better understanding of the underlying factors which in general govern hunger contractions.

In considering the factors which control the motility of the empty stomach Carlson (1916) summarized the work up to that time by concluding that "the gastric hunger mechanism is primarily automatic or independent of blood changes as well as of central nervous influences; but in the normal individual chemical changes of the blood as well as nervous impulses from the brain and spinal cord augment or decrease this primary automatism in a way to correlate it with the needs of the organism."

That the peripheral gastric hunger mechanism is primarily automatic has been substantiated by the observation of comparatively close motor agreement in the isolated stomach and the main stomach noted by Robins and Boyd (1923) for the Heidenhain pouch, Bercovitz (1925) for the denervated Heidenhain pouch, Lim and his co-workers (1927) for the vivi perfused stomach and by Farrell and Ivy (1926) working with the completely denervated isolated gastric pouch. The same experiments may also suggest a control of the hunger contractions by a substance (hormone) present in the blood.

That hunger contractions may be under hormone control is also suggested by the observation of Luckhardt and Carlson (1915) that gastric motility in normal dogs is increased by intravenous injections of blood taken from starved or diabetic dogs. The observations on starved dogs were confirmed by Tschukitschew (1929) who further reported that if blood is drawn from the starved dog during a gastric rest period it will augment the gastric motility of the recipient, but on the other hand, if the donor's

stomach is active at the time the blood is drawn the injection will inhibit the activity of the recipient's stomach. This suggests a stimulatory and depressant hormone.

The idea of a modification of the composition of the blood which would normally control hunger contractions was put into more definite form by the suggestion of Luckhardt that hunger contractions were increased by fasting, moderate exercise, hemorrhage, exposure to cold, phlorhidzin or diabetes because in these conditions there is a decrease in the blood sugar concentration, in the glycogen reserve or in the ability of the body to use carbohydrates. This conclusion was supported by the experiments of Bulatao and Carlson (1924) who observed that insulin injected into dogs increased gastric motility while intravenous injections of dextrose produced inhibition. These authors suggested that the influence on gastric motility of hypoglycemia and of intravenous administration of dextrose is due to a change in the energy metabolism of the gastric motor tissues.

THE HEIDENHAIN POUCH. The normal motility of the Heidenhain pouch has been studied by Robins and Boyd. We have in general corroborated their findings regarding the typical motility exhibited by this stomach preparation during a period of fasting.

Action of insulin and of dextrose on the motility of the Heidenhain pouch. We selected 6 normal dogs each weighing about 15 kgm. and in these animals prepared Heidenhain pouches in the standard manner. At intervals during the three months following the operations on these animals we performed 51 experiments of the following nature. In animals fasting 18 to 24 hours and comfortably lying on a pad we recorded motility from the Heidenhain pouch and sometimes also from the main stomach by rubber balloons attached to water manometers. The balloon was introduced into the main stomach by way of the esophagus. Using a pressure of 5 cm. water in the system we always noted the spontaneous activity of the pouch described as the fundamental rhythm of the pouch by Robins and Boyd. On some occasions we used the nipple method of Templeton (1928) and obtained records similar to those obtained with the balloon method. After making a control record for about 30 minutes we injected insulin either subcutaneously or intravenously in doses of 16 to 80 units (20 to 40 units gave the most satisfactory results). The record was continued without interruption until the animal went into convulsions (usually about 1½ to 2 hours after insulin) in which event dextrose was administered intravenously and the record continued for about 45 minutes longer. In cases where convulsions did not develop the record was continued for about 2½ hours following the insulin injection usually without subjecting the animal to further experimental procedure but in a few cases dextrose was administered about 2 hours after the insulin injection. The dextrose was adminis-

tered in the form of a 50 per cent solution and was given in amounts of 2 to 50 grams.

The typical effect of insulin on the motility of the Heidenhain pouch is inhibition, which in some cases lasted as long as 4 hours. The tone of the pouch was also depressed slightly in most cases. With doses of 15 to 40 units the inhibition was of three general types: 1, complete disappearance of all motility within 6 minutes (fig. 2). This was the usual effect of a large dose administered intravenously. 2. A moderately rapid inhibition of activity not preceded by stimulation and ending in complete inhibition within 25 minutes. 3. A period of slightly increased motility terminating abruptly in quiescence within 40 minutes. The latter two types of response were usually obtained from subcutaneous injections. After the inhibition was complete, subsequent injections of more insulin did not modify the activity. Doses smaller than 15 units produced inhibition but not complete cessation of motility within the 60 minutes following the injection.

In a number of instances where motility was recorded simultaneously from the pouch and from the main stomach it was noted that the inhibition of the pouch occurred at the same time as the typical augmentation of the main stomach (fig. 1). This indicated that the same primary factor was responsible for both changes. From the experiments performed the impression was gained that it was easier to produce inhibition of the pouch than to obtain augmentation of the stomach by insulin.

In the experiments in which convulsions were obtained and intravenous injections of dextrose given, the inhibition of the pouch continued through the period of convulsions and the period following the dextrose injection but frequently during the 45 minute period following the dextrose administration the pouch activity gradually returned to normal.

In animals which did not exhibit convulsions the subsequent activity was of two types: 1, the pouch remained inhibited in the case of those animals which were found to display marked depression on being released at the end of a 2 to 3 hour period; 2, the motility tended to return to normal in those animals which were released after the same time interval and were found to be apparently in normal condition.

Robins and Boyd describe the characteristic movements of the Heidenhain pouch as consisting of a series of contractions recurring at strikingly regular intervals and consisting of from 2 to 5 contractions which appear as separate peaks without complete relaxation and between these series were contractions of irregular occurrence and strength. When our animals were first used, shortly after the preparation of the pouch they all displayed this typical motility. After having been used several times for the experiments previously described, a gradual modification in the type of activity became evident. The individual contractions in the rhythmically recurring series

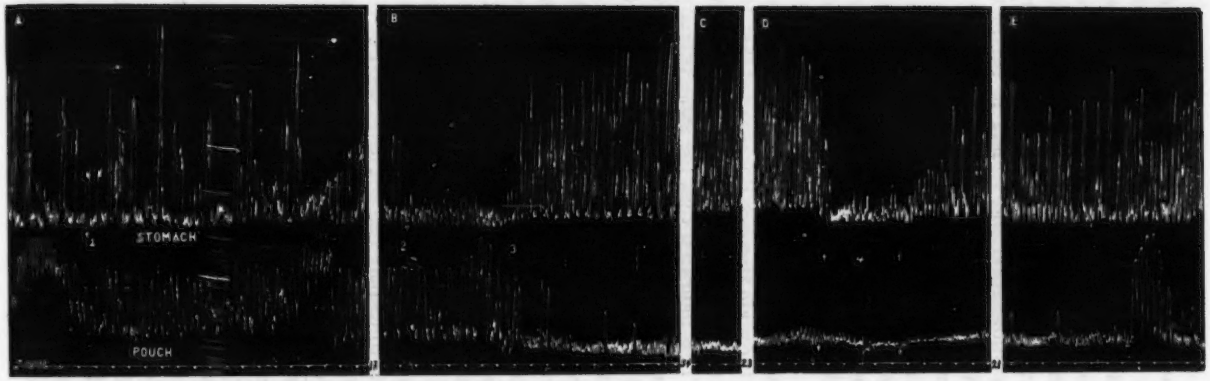

Fig. 1. Record from a dog fasting 18 hours. *1*, injected subcutaneously 16 units of insulin during a normal hunger period; *2*, termination of hunger period in stomach; *3*, thirty-eight minutes after insulin, beginning of stimulation of stomach coincident with inhibition of the pouch; *4*, intravenous injection of 15 grams of dextrose, 132 minutes after insulin; *5*, pouch activity beginning to return 34 minutes after dextrose.

became shorter, the relaxation between contractions became more complete and the isolated contractions became more frequent and of greater amplitude. Finally the rhythmically periodic type of activity was entirely replaced by brief periods of incomplete tetany occurring at frequent but irregular intervals and having short contractions interspersed between them (fig. 3).

At first we were inclined to ascribe this change from the normal type of activity to the fact that our animals tended to become emaciated during the course of the investigation for it is known that the development of emaciation in normal dogs frequently leads to a modification in the type of hunger contractions. However, one of our animals which had been used for

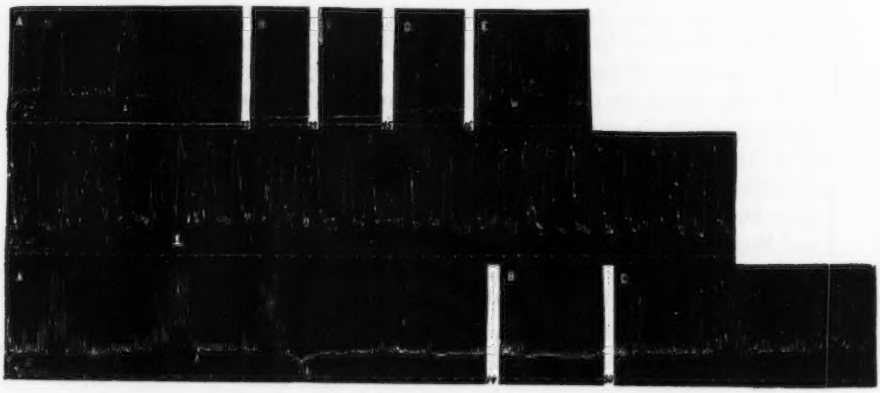

Fig. 2. Record of spontaneous pouch activity, dog fasting 22 hours. *I*, intravenous injection of 16 units of insulin; *E*, return of activity 140 minutes after insulin injection.

Fig. 3. Record of spontaneous pouch activity, dog fasting 20 hours. *I*, intravenous injection of 20 grams of dextrose.

Fig. 4. Record of spontaneous pouch activity, dog fasting 18 hours. *I*, fed 15 grams of cane sugar; *C*, beginning of return of activity, 70 minutes after feeding.

about the same length of time and to which dextrose but not insulin was administered became decidedly emaciated but did not display the change in Heidenhain pouch activity. This suggests that the change may have been due to some direct action of the insulin experimentation on the motor mechanism of the pouch.

Two of the experimental animals in which the change in motility had developed were kept without further experimentation for a period of 2 months but during this interval the motility exhibited no tendency to change toward or further away from the type which developed during the insulin experimentation. This change appeared to be permanent.

Intravenous injection of dextrose not preceded by insulin, in amounts

as great as 50 grams, usually produced in fasting animals no detectable modification in the motility of the Heidenhain pouch (fig. 3) although a simultaneous record showed that the motility of the main stomach was inhibited in the characteristic manner. In some cases records were obtained which indicated that the intravenous injection of dextrose slightly inhibited the motility of the pouch while other records indicated moderate augmentation following the dextrose; in general, however, the dextrose was without effect. Intravenous injections of the same volume of 0.9 per cent sodium chloride solution did not modify the motility of the pouch.

Feeding dextrose or cane sugar in 15 gram amounts to an animal while a record was being made completely inhibited the pouch motility for periods as long as one hour (fig. 4). Robins and Boyd reported that introduction of 100 to 150 cc. of 10 per cent dextrose through a stomach tube did not inhibit motility of the pouch. It seems unlikely that this difference in results was due to the different mode of administration for Robins and Boyd report that allowing the animal to gnaw bones did not inhibit the pouch motility although this procedure would probably produce psychic effect, salivation and swallowing to as great a degree as was produced in our experiments during the administration of sugar by mouth. Since Robins and Boyd were unable to obtain inhibition of the pouch from the introduction of any non-fatty food into the stomach, our results with dextrose if confirmed, would tend to indicate that this substance has a rather unique action on the pouch motility. However, the conclusion of Robins and Boyd that the introduction of non-fatty substances into the stomach did not lead to inhibition of the pouch motility has already been questioned by Bercovitz. This investigator reports that a cold water extract of meat when introduced into the stomach by means of a stomach tube will inhibit the motility of the Heidenhain pouch in an animal from whom the coeliac ganglia have been removed. Bercovitz believes that this finding practically eliminates the contention that inhibition of the pouch is produced only by fatty substances.

We have confirmed the observation of Robins and Boyd that feeding meat to the animal will inhibit the pouch. We observed, however, that if the meat (hamburger) were fed in amount of 10 grams at intervals of 15 minutes, inhibition of the pouch did not occur until a total of about 50 grams had been fed. This observation also suggests that oral feeding *per se* is not the important factor in producing pouch inhibition.

Discussion. It may be that it is the same factor in both cases that is responsible for the failure of the Heidenhain pouch to respond to insulin or to dextrose in a manner characteristic of the normal stomach. Assuming that the causative factor is the same we may infer that *a*, the response of the fundic portion and body of the stomach from which the pouch was made

may be qualitatively different from that of the pyloric portion; *b*, the change is due to a disturbance to the plexus of Meissner and Auerbach; *c*, the change is due to the sectioning of the vagi. (It is generally agreed that in preparing a Heidenhain pouch the sympathetic fibers which reach the stomach along the mesenteric attachment experience very little disturbance.)

The first suggestion was investigated by studying the effects of insulin and of dextrose on the motility of three parts of the stomach of normal fasting dogs. The triple balloon technic was used for this investigation and it was found that the motility was in general similar to that occurring in man as reported by Quigley, Johnson and Solomon (1929). The response of all parts of the stomach to insulin or dextrose appears to be qualitatively the same.

To explain the inhibition of the pouch which resulted from the feeding of dextrose and the absence of inhibition following the intravenous injection of dextrose, we may assume that when dextrose is fed a substance other than dextrose enters the blood stream which is carried to the pouch to produce inhibition, or we may assume as did Robins and Boyd in explaining the inhibiting action of fatty substances that the inhibition is the result of a local reflex from the stomach and duodenum acting through peripheral ganglia reaching the pouch by way of the splanchnics. The former explanation appears to be more satisfactory. The results obtained strongly indicate that dextrose administered intravenously does not act on the peripheral motor mechanism.

It can be assumed that insulin acts either centrally or peripherially. If the site of action is central the opposite effects obtained on the Heidenhain pouch and the main stomach may be related to sectioning of the vagi. If the action is peripheral we can assume that the reversal in effect is due to an alteration in the irritability of the peripheral motor mechanism to the insulin action. The relation of the action of insulin to its effect on the lowering of blood sugar and the evaluation of the relative importance of the central and peripheral mechanisms in the initiation and control of hunger contractions will be reported in a later paper.

SUMMARY

1. In dogs fasting 14 to 40 hours intravenous or subcutaneous injections of 8 to 80 units of insulin inhibit the motility and depresses the tone of the Heidenhain pouch at the same time that the tone and motility of the main stomach is being augmented.

2. Intravenous injections of 2 to 50 grams of dextrose in the form of a 50 per cent solution are usually without effect on the motility of the pouch.

3. Feeding dextrose to the animal inhibits the pouch motility.

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ACTION OF INSULIN ON THE MOTILITY OF THE GASTRO-INTESTINAL TRACT

III. a. Action on the Pyloric Pouch. b. Action on the Stomach Following Double Splanchnicotomy

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In an attempt to extend our knowledge regarding the mechanism by which insulin increases the gastric motility of normal fasting dogs we investigated the action of insulin on the motility of a pouch made from the pyloric portion of the stomach and also on the gastric motility of dogs following splanchnicotomy.

Pyloric pouch. An isolated pouch of the pyloric portion of the stomach was prepared in one dog in the following manner. The stomach was completely severed at the transverse band and both halves of the stomach closed along the lines of incision. The pylorus and first portion of the duodenum were mobilized and the duodenum separated from the pylorus by an incision just below the pyloric sphincter. The cut end of the duodenum was closed and this portion of the duodenum left as a blind tube. A gastro-enterostomy was made between the body of the stomach and the first part of the jejunum. The pyloric sphincter end of the pyloric portion of the stomach was brought to the abdominal wall in a manner suitable for providing an opening to the pyloric pouch. The animal made an uneventful recovery and the motility of the pouch was studied for over three months. The appetite of the animal was at all times excellent.

In performing the operation no attempt was made to obtain a denervated pouch. The maximum amount of blood supply was retained and it was thought that the extrinsic nerves would reach the pouch along with the blood vessels. About two months after the operation the animal was anesthetized with ether, balloons arranged for recording motility were placed in the main stomach and the pouch, the vagi were exposed aseptically in the neck and both the left and right vagi were stimulated electrically with various strengths. From the records so obtained it was concluded that the vagi at this time were not functionally active to the pouch.

During the ether anesthesia, the striking observation was made that the motility of the pouch was exactly the same as before the anesthesia (fig. 1 B) while the motility of the main stomach was inhibited in the char-

acteristic manner. In so far as we can ascertain this is the first time that hunger contractions of the same type have been recorded from the anesthetized and the unanesthetized animal when ether was employed.

Motility of the pyloric pouch was recorded when the animal was fasting for 18 to 50 hours. The balloon method was employed in the same manner as was previously described. Using this technic in 16 experiments it was found that the pyloric pouch always exhibited vigorous activity of the type shown in figures 1A, 1B, and 2. Shortly after the pouch was prepared the typical movements consisted of rhythmically recurring periods of partial tetany of about three minutes' duration occurring about every 35 minutes and having 9 or 10 contractions superimposed upon them. These were separated by individual contractions occurring about every 20 seconds. Immediately after a period of partial tetany the individual contractions were of low amplitude but the succeeding contractions increased in height until after about 15 contractions a maximum was obtained which persisted with slight changes until the next period of partial tetanus occurred (fig. 1A).

About two weeks after the operation the character of the motility changed to a type which persisted throughout the remainder of the investigation. This motility consisted of isolated contractions occurring at intervals of 20 seconds and of 20 seconds' duration, they were very uniform in height (fig. 1B, and 2). The original activity of the pyloric pouch resembled somewhat that described for the Heidenhain pouch by Robins and Boyd (1923) and that which developed later suggests the activity of the denervated Heidenhain pouch as described by Bercovitz (1925). The change in the character of the pyloric pouch motility appeared to indicate a gradual disappearance, probably as a result of the operation, of some factor originally concerned in determining the character of the pyloric motility. The response of the pouch to insulin or dextrose was the same with either type of spontaneous activity.

Effect of insulin and of dextrose injections on the motility of the pyloric pouch. As the result of 16 experiments performed on this animal, it was determined that in general the subcutaneous or intravenous injections of insulin in amounts from 16 to 80 units did not modify the motility or tone of the pyloric pouch during the following 2 to 3 hour period. A few records were obtained in which a slight inhibition of short duration appeared to follow the intravenous injection of insulin in amounts exceeding 40 units. The occurrence of hypoglycemic convulsions apart from the body movements which they induced also failed to modify the pouch motility. Intravenous injections of dextrose in amounts up to 25 grams regardless of whether or not they followed the administration of insulin were practically without effect on the pouch tone or activity (fig. 2).

Discussion. Since both dextrose and insulin affect the motility of the

Fig. 1. A. Typical spontaneous activity from pyloric pouch 4 days after pouch was prepared. Animal fasting 20 hours.
B. Spontaneous activity of pouch during ether anesthesia.

Fig. 2. Typical spontaneous activity of pyloric pouch 4 weeks after the pouch was prepared. Animal fasting 20 hours. *1*, subcutaneous injection of 20 units of insulin; *2*, tremors; *3*, mild convulsions; *4*, severe convulsions; *5*, intravenous injection of 15 grams of dextrose. The amount of change in activity (apart from convulsive movements) shown in this record was frequently noted in control records.

pyloric portion of the stomach of normal fasting dogs to such a marked degree, the absence of effect from these two substances becomes more striking. If we assume that the pouch was completely denervated and that insulin and dextrose modify gastric motility by some central action, the results obtained can be understood. Our results do not preclude the possibility that insulin and dextrose normally act peripherally. If this were the case, however, we must assume that in the pyloric pouch the operative procedure had produced a marked reduction in the irritability of the peripheral motor mechanism.

SPLANCHNICOTOMIZED ANIMALS. *Methods.* In ten normal dogs each weighing about 15 kgm. the splanchnic fibers were sectioned in the thoracic cavity by removing about one inch from the nerve just above the diaphragm. Necropsy of these animals established the fact that all of the splanchnic fibers on both sides had been sectioned.

Fifty experiments were performed on these animals during the interval of three days to three weeks after the operation. The experiments were designed to show the effect of insulin and of dextrose injections on the motility of the stomach during fasting. In animals fasting for 20 to 44 hours and lying comfortably on pads, records of the gastric motility were obtained by the balloon method. The balloons were introduced into the stomach by way of the esophagus.

In a few cases determinations of the blood sugar concentration were made preceding the insulin injection and again at the time of hypoglycemic convulsions. Determinations were made at times by the method of Somogyi (1926) and on other occasions by the "true blood sugar" method of West, Scharles and Peterson (1928). (We are indebted to Mr. E. I. Solomon for these determinations.)

Results. Our dogs after splanchicotomy normally displayed the vigorous and continuous type of hunger contractions which Carlson (1916, p. 153) has described as typical for such animals.

The characteristic effect of subcutaneous injections of 20 units of insulin was a rise in gastric tone and an increase in motility beginning about twenty to forty minutes after the injection. This increase in tone and motility continued until it reached a maximum about fifteen minutes later. At this time the stomach usually displayed type III activity. From here on, the motility and tone continued at about the same degree, or more frequently, decreased slightly until approximately twenty minutes later when severe struggling or convulsions occurred.

In those animals in which convulsions developed (fig. 4) the tone and motility were maintained fairly well until dextrose was injected to relieve the convulsions. The intravenous injection of 15 grams of dextrose at this phase of the experiment was followed by a marked decrease in gastric tone and motility. This inhibition was of short duration for within five minutes

the tone and motility had begun to increase again. This usually continued until a second convulsion occurred about one hour following the first convulsion. If 30 grams instead of 15 grams of dextrose were administered the second increase in activity and the second convulsion did not occur.

With those animals in which severe struggling but no convulsions occurred during the period of marked gastric response induced by insulin (fig. 3) the record following the period of struggling followed one of two general routes. In some animals the period of struggling had very little effect on the gastric activity (fig. 4, 7) and tone and motility were maintained until convulsions occurred about ten to twenty minutes later. On other occasion and under conditions which appeared to be identical with those just described, the period of struggling was followed by a marked decrease in gastric tone and motility. If dextrose were injected at this time it produced little or no further inhibition of the stomach. In some cases the inhibition was transient being succeeded by a return to a condition of high tone and motility terminating in convulsions about 50 minutes following the period of struggling. In other experiments the gastric inhibition persisted and convulsions developed while the tone and motility of the stomach were depressed (fig. 3). In this event, the intravenous injection of 15 grams of dextrose terminated the convulsions but was without effect on the stomach activity.

Of the modifications in gastric activity described above the only one noted in control experiments where no injections were made was the inhibition produced by struggling. In normal dogs we have obtained records where hypoglycemic convulsions occurred not immediately preceded by marked gastric activity and we have also obtained records where struggling inhibited the insulin gastric response.

The intravenous injection of 15 grams of dextrose (not following insulin) produced in splanchnicotomized dogs a complete inhibition of the stomach. Within 30 minutes, however, the activity had returned to the degree exhibited preceding the injection. This injection of dextrose produced no demonstrable effect on the response to insulin if this insulin was injected when activity had returned to normal. The increase in gastric tone and motility and the hypoglycemic convulsions occurred in the usual manner.

In splanchnicotomized dogs blood sugar determinations made by the method of Somogyi gave values between 103 and 125 mgm. per cent for animals fasting 20 hours preceding the injection of insulin and values around 30 mgm. at the time of convulsions. A dog which was subjected to the same experimental procedures but in which convulsions did not develop and the increase in gastric activity was only slight had a blood sugar value of 65.8 three hours after the insulin injection.

By the method of West, Scharles and Peterson animals fasting 20 hours had a blood sugar of 78.0 to 81.6 mgm. per cent before insulin and 8.0 to

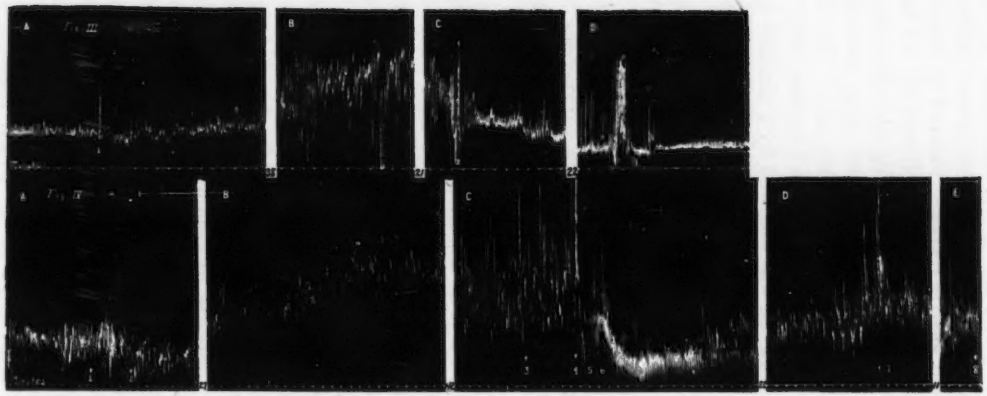

Fig. 3. Record from stomach following double splanchnicotomy, dog fasting 20 hours. *1*, injected subcutaneously 20 units of insulin; *2*, severe struggling; *3*, convulsions, injected intravenously 7.5 grams of dextrose.

Fig. 4. Record from stomach following double splanchnicotomy, dog fasting 20 hours. *1*, true blood sugar 81.6; *2*, injected subcutaneously 20 units of insulin; *3*, profuse salivation began; *4*, severe convulsions; *5*, true blood sugar 6.0; *6*, intravenous injection of 15 grams of dextrose; *7*, severe struggling without gastric inhibition, *8*, convulsions.

19.2 mgm. at the time of convulsions. With dogs fasting 44 hours the values were 60.0 to 70.0 mgm. before insulin and 3 to 12 at the time of convulsions.

DISCUSSION. The records of gastric activity obtained after injecting insulin in fasting splanchnicotomized dogs resembled those obtained under similar conditions from normal animals. In general, however, the injection of insulin appeared to give rise to a slightly greater increase in gastric activity and tone in splanchnicotomized dogs than in the normal animal. There are three possible reasons why this augmentation was not more marked: 1, the impulses over the sympathetic fibres induced in normal dogs appear to play a minor rôle in determining the type of stomach activity induced by insulin; 2, the gastric response to insulin always displays marked individual variations; 3, the stimulation of the sympathetic system may be peripheral and not be altered by splanchnicotomy. The response to intravenous injections of dextrose was also similar in the two groups of animals.

Our results do not appear to inform us whether the site of action of insulin on the sympathetic system is central or peripheral. Either the splanchnics play a very minor rôle in the response of the normal fasting stomach to insulin and dextrose or else the action of these substances is on the peripheral portions of these nerves and is but little disturbed by the splanchnicotomy.

At present we are at a loss for an explanation of the inhibition of the gastric insulin response which occurred on several occasions following struggling. That the inhibition was not the result of an increase in blood sugar is apparently shown by the fact that hypoglycemic convulsions could later occur while the gastric inhibition still persisted. It is possible that the inhibition was produced by inhibitory impulses over the vagi.

SUMMARY

1. The motility of a pouch made from the pyloric portion of the stomach was not appreciably altered by injections of 16 to 80 units of insulin or the intravenous injection of dextrose up to 30 grams.

2. The action of insulin and of dextrose on the gastric motility in double splanchnicotomized dogs while fasting is similar to but slightly greater than that displayed by normal animals.

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ACTION OF INSULIN ON THE MOTILITY OF THE GASTRO-INTESTINAL TRACT

IV. Action on the Stomach Following Double Vagotomy

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A study by Quigley, Johnson and Solomon (1929) of the activity produced by insulin in three parts of the human stomach, demonstrated that the pyloric end of the stomach was stimulated to a greater degree than the remaining portions of the stomach. Since electrical stimulation of the vagi likewise produces its greatest motor effect on the pyloric region, it was suggested that the gastric response to insulin might involve central vagal stimulation. Our experiments with the triple balloon in normal dogs and our study of the Heidenhain pouch also emphasized the importance of the vagus in this phenomenon. We have therefore investigated the action of insulin on the hunger contractions of dogs following double vagotomy.

Methods. The vagi were sectioned above the diaphragm in five normal dogs. The results of the operations were subsequently established by examination of the vagi at necropsy or by putting a balloon in the stomach of the anesthetized animal, exposing the vagi aseptically in the neck, exciting electrically and noting the absence of contractions of the stomach. Our animals displayed the type of hunger contractions, which have been described by Carlson (1916, p. 156) as being typical of vagotomized animals.

During the three months period which followed the operation 64 experiments were performed to determine the action of insulin and of dextrose injections on the motility of the stomach during fasting. With the animals comfortably lying on pads, records of stomach activity were made by the balloon method. The balloons were introduced into the stomach by way of the esophagus in four dogs and through a gastrostomy opening in the remaining animal. Records were made when the animals were fasting 18 to 44 hours, insulin was injected intravenously or subcutanously in doses from 8 to 80 units and dextrose was injected intravenously in the form of a 50 per cent solution. Since the stomach of vagotomized dogs empties slowly, the animals were sometimes given a liter of water by stomach tube 4 or 5 hours preceding the experiment to aid in emptying the

stomach of food residues. The experiments were conducted in a manner similar to that described in our previous papers.

Results. In general the injection of insulin produced gastric inhibition in vagotomized dogs. If administered during a period of gastric activity inhibition occurred either within four minutes (fig. 1) or after about 15 to 45 minutes (fig. 2). The former result was that usually obtained from intravenous injections while subcutaneous injections usually produced

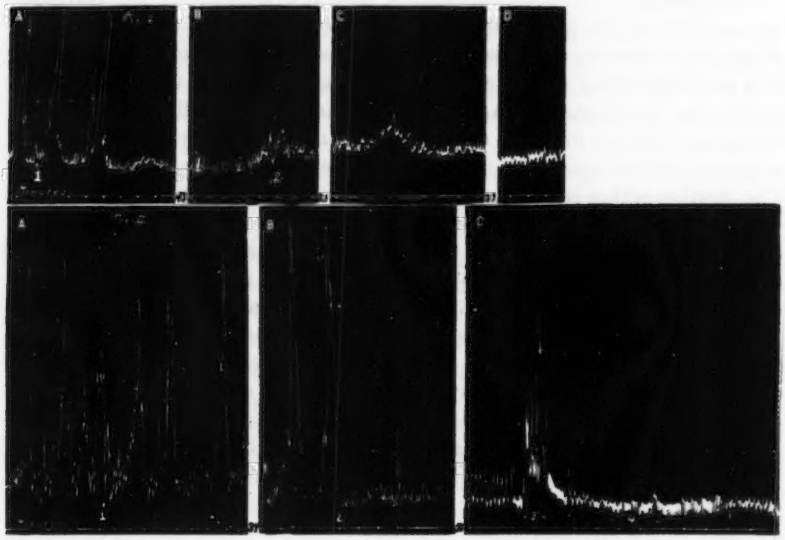

Fig. 1. Record from stomach following double vagotomy, dog fasting 25 hours. *1*, intravenous injection of 16 units of insulin produced complete gastric inhibition in 4 minutes; *2*, increase in tone 54 minutes following insulin.

Fig. 2. Record from stomach following double vagotomy, dog fasting 23 hours. *1*, subcutaneous injection of 20 units of insulin; *2*, inhibition of stomach 47 minutes after insulin; *3*, severe convulsions; *4*, intravenous injection of 20 grams of dextrose.

the more delayed inhibition. The inhibition lasted for several hours and was not modified by subsequent injections of insulin or by increasing the pressure in the balloons. In some cases an increase in tone but not in motility occurred about 50 minutes after the injection of insulin and persisted for about 25 minutes. It had the appearance of a hunger period attempting to break through the inhibition. If the primary injection of insulin were made during a gastric rest period no return of activity occurred during the remainder of the experiment, frequently for the subsequent 4 hour period. Gastric inhibition continued during the interval preceding

and following hypoglycemic convulsions. Intravenous injections of dextrose in amounts from 10 to 30 grams at this time usually did not modify the gastric activity. With vagotomized dogs the experiment was frequently interrupted by vomiting on the part of the animal 1½ to 2 hours following the administration of insulin.

The intravenous injection of dextrose in doses from 10 to 30 grams (not preceded by insulin administration) usually did not modify the gastric activity of vagotomized dogs.[1]

DISCUSSION. The observation that insulin injections do not increase the motility of the empty stomach of vagotomized dogs while the other symptoms of insulin overdosage (convulsions, etc.) occur in these animals in a normal manner suggests that the vagi are concerned in the action of insulin on the stomach. Apparently insulin acts centrally to produce a discharge of motor impulses over the vagi but in vagotomized animals the impulses can not reach the stomach because of the surgical interruption. The insulin action may be on the dorsal vagus nuclei which Laughton (1929) has shown can be stimulated electrically to produce strong contractions of the stomach. On the other hand it may suggest that insulin acts peripherally, but the sectioning of the vagi had reduced the irritability of the tissue (vagus endings) on which it would act in normal animals. The former explanation appears to be more satisfactory. The gastric inhibition from insulin noted in dogs having the vagi sectioned may be due to the insulin stimulating the sympathetic system and leading to the discharge of inhibitory impulses to the stomach.

The effect of insulin on the gastric motility in the normal animal may be explained by assuming that the injection of insulin leads to a discharge of augmentatory impulses over the vagi which increase both the tone and motility of the stomach and of inhibitory impulses over the sympathetic fibers to the stomach to oppose the vagal effect. They would lead to a final action on gastric motility which is the algebraic sum of these two groups of impulses. Usually the influence of the vagi predominates and augmentation of activity occurs.

On the basis of this explanation, inhibition of the Heidenhain pouch is produced by insulin for the vagus fibers to this stomach preparation are sectioned leaving the inhibitory impulses unopposed. If the sympathetic and vagus fibers to a portion of the stomach both fail to carry the usual type of impulses, it would be expected that insulin would be without action on this type of stomach preparation. This may explain the results obtained with the pyloric pouch. This mechanism may also be involved

[1] We have incidentally noted that injections of atropine or of epinephrin will inhibit the gastric motility induced in normal dogs by insulin. In dogs under barbital anesthesia we have obtained no gastric motility either spontaneously or after the intravenous injections of 40 to 500 units of insulin.

in the inhibition of the insulin gastric response which is produced by the injection of atropine for the paralysis of the vagi would leave the sympathetics unopposed. In the case of animals with the splanchnics sectioned the gastric activity after insulin was slightly greater than in normal animals as our theory suggests that it should be.

Since our results indicate that the gastric insulin response is so dependent on the integrity of the vagi it may be that insulin injections could be used as a test of the functional activity of the vagus fibers to the stomach.

In considering the mechanism of the insulin action on gastric motility we encounter the question of whether the results are due to insulin *per se* or are related to the hypoglycemia and modified cell metabolism which it induces. The results obtained by Bulatao and Carlson (1924) as well as our own work appears to favor the latter conclusion. The marked effect which we obtained on the Heidenhain pouch and the vagotomized stomach 4 minutes after the intravenous injections of large doses of insulin probably does not contradict this statement if it can be shown that during this time interval the intravenous injection of insulin would produce a definite lowering of the blood sugar. Bodansky and Simpson (1927) noted a fall of 3 mgm. per cent in 5 minutes and as much as 33 mgm. per cent in 9 minutes following the intravenous injection of 2 units of insulin to normal human subjects. On the other hand the effect of insulin on cell metabolism may occur a significant interval of time before a pronounced modification of the blood sugar is demonstrable. Our prompt results with the Heidenhain pouch were not due to the sensitivity of this preparation being greater than that of the normal stomach for the insulin effects were frequently obtained simultaneously on the two structures. Cannon, McIver and Bliss (1924) have shown that a discharge of adrenalin occurs when the blood sugar after insulin falls to about 70 mgm. per cent. This adrenalin discharge may have been involved in the inhibition of the Heidenhain pouch or vagotomized stomach which we have observed. The prompt inhibition of the gastric response to insulin which follows the intravenous injection of dextrose noted in normal or splanchnicotomized dogs may be due either to the relief of the hypoglycemia or to a binding of the insulin in the body by the dextrose.

Intravenous injections of dextrose when given either before or after the injection of insulin inhibit the motility of the normal stomach or the stomach after splanchnicotomy. This inhibition is apparently by way of the vagus for it does not occur in the Heidenhain pouch or in the stomach after vagotomy.

The observation that dextrose injected intravenously has no effect on the Heidenhain pouch while dextrose given orally inhibits the pouch suggests that the latter inhibition is not the result of dextrose in the blood but of dextrose in the gastro-intestinal tract. The observation of Quigley,

Johnson and Solomon that dextrose in the stomach does not inhibit the insulin gastric response while dextrose in the duodenum does lead to inhibition indicates that it is the duodenum which is concerned in the above inhibition. It appears that dextrose in the duodenum liberates a substance other than dextrose which is carried by the blood to the Heidenhain pouch to produce inhibition, or the dextrose may produce an inhibitory reflex from the duodenum to the pouch. This also suggests that the blood sugar level is not the factor determining normal hunger contractions.

With the vivi-perfused stomach Lim and Necheles (personal communication) have frequently but not invariably noted that the intravenous administration of insulin to the unanesthetized perfuser dog produced an immediate but transient augmentation of the motility in the isolated stomach. Under similar conditions dextrose occasionally produced an immediate but transient gastric inhibition. Since we have not obtained inhibition from dextrose or augmentation from insulin in stomachs with the vagi sectioned, these results appear to be slightly at variance with our conclusions. The same statement also applies to the observation of Farrell and Ivy (1926) that the motility of the transplanted gastric pouch is inhibited in 5 minutes by the intravenous injection of "comparatively large" amounts of dextrose. (In the normal stomach the inhibition from intravenous injections of dextrose appears immediately.)

Our conclusions receive support from the observations of Stucky, Rose and Cowgill (1928) that the gastric tone and motility were low in vitamin-B deficient dogs. These animals also displayed a greatly reduced gastric response to insulin. This suggests to us that the vagus activity in these animals was depressed. These authors conclude that they were unable to demonstrate in their animals any direct relationship between gastric motility and blood sugar level.

In normal animals intravenous injections of dextrose produce only a transient gastric inhibition, and activity returns while the blood sugar and especially the glycogen reserve is well above normal. On the other hand the gastric activity produced by insulin does not appear until the blood sugar is below the values occurring normally. These facts seem to indicate that physiological variations in the blood sugar level or glycogen reserve are not the essential factors in the control of the normal hunger contractions. Abnormally great variations in the amount of blood sugar may modify gastric motility; high blood sugar leading to inhibition by way of the vagi; low blood sugar leading to augmentation by simulation of the vagi though opposed by a simultaneous stimulation of the sympathetic mechanisms.

Our results tend to emphasize the importance of a hormone in the primary control of motility in the fasting stomach and to indicate that this

hormone is not the blood sugar. They further emphasize the importance of the central nervous system in the modification of this motility.

SUMMARY

1. In dogs having the vagi sectioned insulin inhibits gastric hunger contractions while intravenous injections of dextrose are practically without effect on the motility of such a stomach.

2. The mechanism by which insulin and glucose modify gastric motility is considered.

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ACTION OF INSULIN ON THE MOTILITY OF THE GASTRO-INTESTINAL TRACT

V. a. Action on the Human Duodenum. b. Action on the Colon of Dogs

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Since Bulatao and Carlson (1924) have shown that injections of insulin increase the gastric activity of normal fasting dogs, and Quigley, Johnson and Solomon (1929) have demonstrated a similar phenomenon in man, it was decided to extend the investigation to other portions of the gastro-intestinal tract. The observation of Quigley, Johnson and Solomon that the subject of an experiment was frequently able to predict from his sensations that the gastric response would shortly make its appearance suggested that another portion of the tract was in activity at that time and was responsible for the sensations being perceived. The observation of Ivy and Vloedman (1925) of a close relation between the motor activity of the duodenum and the stomach of fasting man and dogs suggested that the duodenum might be the source of the sensations. The relations between motility of the stomach and colon noted by Welch and Plant (1926) suggested that the investigation of the effect of insulin on the latter organ was also warranted.

Action of insulin on the motility of the human duodenum. *Methods:* Six experiments were performed on a normal man to determine the action of insulin on the motility of the human duodenum. Activity of the intestine was recorded with either a single or a double balloon in the duodenum and this activity was correlated with the response of the stomach by making simultaneous records from a single or a triple balloon placed in the stomach.

The triple balloon used in the stomach was the one described by Quigley, Johnson and Solomon. The double balloon used in obtaining records from the duodenum was prepared in a similar manner but differed from it in having only two balloons and two tubes and being considerably smaller. The device was further modified in that the tube to the lowest balloon was extended through the bottom of the balloon for about two inches; this portion was filled with mercury and suitably tied off. The flexible weight aided materially in the passage of the double balloon through the alimen-

tary tract and also permitted the position of the balloon being determined by means of a fluoroscopic examination. The tubes from the balloons were connected to water manometers for recording changes in pressure within the viscus.

With the subject fasting for 18 hours the double balloon was swallowed into the stomach and the subject proceeded with his usual activities. Gradually during the ensuing two hour period about 18 inches more of the rubber tubing was swallowed and small amounts of water were taken at frequent intervals. Towards the end of this period a fluoroscopic examination usually disclosed the mercury weight several inches past the pyloric sphincter. The stomach balloon was then swallowed. The position of the balloons in the duodenum was always determined at the beginning and termination of the experiment but records were also made with the subject lying on the x-ray table, permitting frequent examinations throughout the experiment.

We did not find it necessary to follow the procedure suggested by Ivy and Vloedman of swallowing the duodenal balloon the night preceding the experiment. The method described above was always, in our experience, entirely satisfactory.

The subject was not annoyed or nauseated by the balloons or tubes and with the exception of the unpleasant effects produced by the insulin was entirely comfortable during the experiment. Having been the subject for a number of previous experiments on gastric motility he was well fitted to make observations on subjective sensations.

With the subject fasting about 20 hours, lying on a pad and with the balloons in place, control records were made. We found that the duodenal record was materially modified by changes in the balloon pressure and therefore we gave this point especial consideration. We employed the minimum pressure which would give a satisfactory record, usually equivalent to about 2 cm. of water. Insulin in doses from 12 to 20 units was then administered subcutaneously and the record continued. The subjective and objective reactions of the subject were noted and on several occasions attempts were made to modify the record by introducing dextrose into the stomach or administering atropine subcutaneously.

Results. During our control experiments we have confirmed the observation of Ivy and Vloedman that the hunger periods of the stomach and duodenum are related. Peristaltic waves in the stomach almost always passed over into or reappeared in the duodenum and the duodenal activity was also peristaltic. This was strikingly illustrated in records obtained with the triple balloon in the stomach and with the double balloon in the duodenum. The hunger period terminated in the duodenum in a period of tetanus lasting for 2 to 4 minutes (fig. 1). The termination of the duodenal hunger period usually occurred about 3 minutes after the termination of the

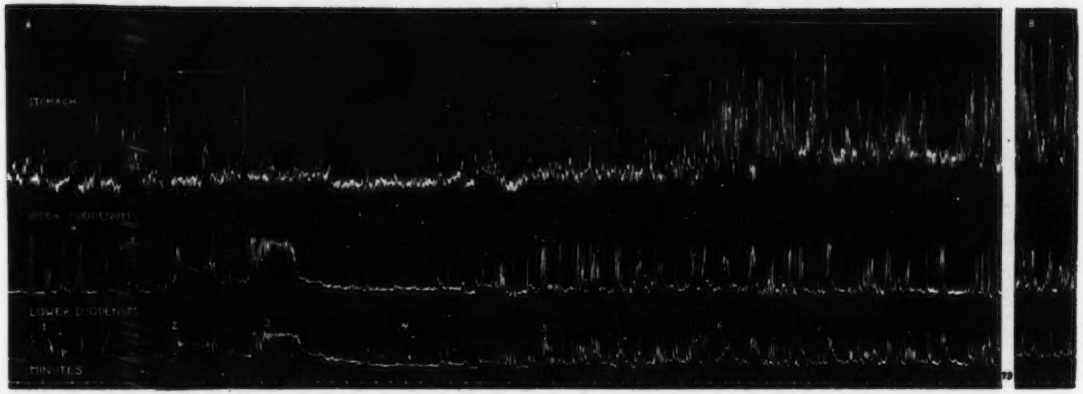

Fig. 1. Record from man fasting 20 hours, lower balloons in midportion of duodenum. *1*, subcutaneous injection of 16 units of insulin; *2*, borborygmi; *3*, termination of a hunger period; *4*, no sensations of hunger; *5*, mild but prolonged sensation of hunger, not unpleasant, related to duodenal activity. Sensation deep and located in the region of the navel; *6*, typical sensation of hunger localized to skin near zyphoid process, unpleasant; subject restless, sweating and weak.

B, 145 minutes after insulin.

gastric hunger period. Our records of the stomach and duodenum do not show a relaxation preceding the contraction in these structures. The activity appears to be katastalsis rather than peristalsis.

The frequency of duodenal contractions in our experiments was much lower than has been reported by other investigators. Whether this was the result of a different experimental procedure or an individual characteristic of our subject, we can not state. We were able to increase the frequency of duodenal contractions decidedly by raising the pressure in the duodenal balloons.

During the control period movements of the duodenum usually produced little sensation of hunger in comparison with similar contractions of the stomach. On several occasions borborygmi were heard and definitely related to contractions in the duodenum. We were frequently able to note a pulse record on our duodenal tracings.

The duodenal response to the injection of insulin always occurred about 15 minutes preceding that of the stomach. The response of the duodenum consisted of a gradual increase in frequency of the contractions while the tone was usually unaltered. These contractions soon became more frequent than was seen in the control period. If the stomach was quiescent or displaying very little activity at this time, as was usually the case, these contractions were accompanied by an ill defined but not unpleasant sensation of mild hunger localized about 2 cm. above and 3 cm. to the right of the navel. The subject readily learned to recognize this sensation and to correlate it with duodenal activity. We are not in agreement with Ivy and Vloedman in their contention that hunger contractions in the duodenum are due "only to the passage of the gastric hunger contractions or their effect, from the stomach down to the duodenum." We obtained vigorous duodenal contractions during a period of complete gastric rest. Since the sensations described above were noted during this interval we believe that our observations settle the question raised by these authors regarding the rôle of the duodenum in the genesis of the hunger sensation. We are in a position to state that in this subject duodenal contractions in the absence of gastric contractions gave rise to a sensation of hunger. It appeared very likely that the subject who had received insulin was able to predict several minutes in advance that the typical gastric response was about to occur because of the activity in the duodenum which preceded the gastric activity.

When the gastric response to insulin began, the duodenal sensations were replaced by the unpleasant sensations of typical hunger. The location of the sensations was also shifted to the region of the stomach.

During the control periods the contractions of the stomach and duodenum were almost always peristaltic and the gastric wave in practically every case appeared to pass over the sphincter to be succeeded by a wave

in the duodenum. The contractions in the two portions of the alimentary tract were of the same frequency. After the gastric response to insulin had begun the activity in the two regions still appeared to be peristaltic but the gastric contractions were several times as frequent as those of the duodenum. Apparently many of the gastric contractions were blocked from the duodenum or the duodenal irritability was not sufficiently high to respond at the increased frequency.

On one occasion a rest period occurred in the stomach and duodenum about 10 minutes after the beginning of the gastric response. This lasted for three minutes and the activity which immediately succeeded this was definitely in the order: duodenum, pyloric, middle and cardiac region of the stomach. During the rest period the sensation of hunger was absent and there was no feeling of nausea either at this time or during the interval of the reversed wave which followed it.

The characteristic response of the duodenum to insulin was of long duration and was in this respect similar to the response of the stomach (Quigley, Johnson and Solomon). Activity persisting for several hours has been recorded from both organs. The duodenal activity induced by insulin differed from the spontaneous activity only in the increased frequency of contractions and the prolonged duration of activity which followed the insulin.

We have corroberated the finding of Quigley, Johnson and Solomon that the insulin response of the stomach may be completely inhibited by the subcutaneous injection of atropine. We have also noted as they did the occurrence of waves of reversed peristalsis appearing in the stomach as motility began to return following the atropine injection. In addition to this we have noted that the duodenum was also inhibited. This duodenal inhibition occurs more promptly and is of longer duration than the inhibition shown by the stomach. The reversed peristalsis noted in the stomach did not appear in the duodenum in our experience; in fact, the duodenum remained entirely quiescent during the 20 minute period which followed the subcutaneous injection of 1 mgm. of atropine. At this time the condition of the subject caused us to discontinue the experiment.

We have also inhibited a vigorous gastric and duodenal response to insulin by giving 100 cc. of water containing 25 grams of cane sugar and collapsing the balloons for 15 minutes to permit the free passage of the sugar through the intestine. The inhibition so obtained was much more complete for the stomach than for the duodenum. A control experiment in which water alone was administered did not result in such inhibition.

ACTION OF INSULIN AND DEXTROSE ON THE MOTILITY OF THE COLON. *Methods.* In two normal dogs we mobilized the appendix and in closing the abdominal wall left the tip of the appendix outside. This was subsequently opened to form a colostomy after the abdominal incision had

Fig. 2. Record from dog fasting 22 hours. *1*, injected subcutaneously 20 units of insulin; *2*, convulsions; *3*, injected intravenously 15 grams of dextrose.

healed. On these animals 24 experiments of the following type were performed. The dogs were fasted for 18 to 44 hours and then placed comfortably lying on pads with a balloon in the stomach, by way of the esophagus, and a second balloon in the colon. Control records were made and then insulin in doses from 16 to 40 units was administered subcutaneously without interruption of the record. When convulsions developed dextrose was administered intravenously and the record continued.

Results. We obtained records during the control periods which resemble those obtained by Plant and his co-workers.

The normal activity of the colon consisted of periods of increased tone with superimposed contractions occurring at comparatively uniform intervals. These periods of activity were separated by intervals of almost complete quiescence. Following the admistration of insulin the intervals became shorter until by the time the increased gastric motility became evident the contractions of the colon had become very vigorous and a high state of tone was maintained. This condition persisted as the gastric activity increased and the hypoglycemic convulsions developed but was terminated by the intravenous injection of dextrose given to relieve the convulsions. The inhibition of the colon which followed the administration of 15 grams of dextrose was much less complete than in the case of the stomach, for the periods of colonic activity continued to appear at intervals about one half as long as those which proceeded the insulin administration (fig. 2). The colon appeared to be more uniformly stimulated by insulin than was the stomach, for records were obtained displaying a marked increase in colonic activity but showing an indefinite response by the stomach. The increased motility of the colon did not lead to defecation for this was absent unless convulsions occurred.

As a tentative explanation of the mechanism by which the observed increase in motor activity of the duodenum and colon following the injection of insulin is produced, we suggest that insulin administration gives rise to an increase in the augmentatory impulses transmitted to these organs over the vagi and nervi erigens.

We take pleasure in expressing to Dr. A. J. Carlson our appreciation of facilities and helpful criticism which he so kindly offered.

SUMMARY

1. Duodenal activity has been followed in man by the double balloon method and has been related to gastric activity.

2. Subcutaneous injection of 12 to 20 units of insulin was followed by an increase in duodenal activity similar to that which occurred in the stomach.

3. Duodenal hunger contractions can occur in the absence of gastric motility and this duodenal activity can give rise to hunger sensations.

4. Since the duodenal response to insulin preceded that of the stomach it may have given rise to sensations which enabled a subject to predict that a gastric response was about to occur.

5. The response of the duodenum as well as of the stomach has been inhibited by the subcutaneous injection of atropine or the oral administration of cane sugar which is allowed to enter the intestine.

6. Insulin injections produced in dogs an increase in colonic activity which closely paralled that exhibited by the stomach.

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STUDIES ON THE PHYSIOLOGY OF SECRETIN

II. The Effect of Purified Secretin on the Blood Sugar

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Many investigators have observed a fall in the blood sugar following the intravenous injection of secretin preparations—Santos (1925); Zunz and LaBarre (1929); Penau and Simonnet (1925). Mellanby (1928) denies such an effect. A full bibliography on the effects of secretin on the blood sugar is to be found in the paper by Zunz and LaBarre (1929).

In a previous paper a method of preparing secretin of great activity was described (Still, 1929). This secretin was found to have no effect on the blood sugar. The following was the plan of experimentation:

In the first series of experiments, dogs under barbital (225 mgm. per kilo) were used. The pancreatic duct was cannulated, the saphenous vein prepared for injections and femoral artery for collection of samples of blood for subsequent analysis. A control sample of blood was taken, then the pancreas was kept in a state of activity for about three hours by injecting 0.1 mgm. of purified secretin per kilo each thirty minutes. Samples of blood were drawn each 30 minutes for analysis. The glucose was determined by the method of Folin and Wu. Six such experiments did not give any evidence of an insulin-like property of the secretin preparations used.

It was believed that unanesthetized animals might lend themselves better to the demonstration of the alleged insulin-like properties of secretin. The following experiments were carried out.

Six dogs of about 15 kgm. weight were chosen and placed on a diet consisting of 250 grams meat, 250 cc. milk and 5 grams bone meal per day. Dogs 1, 2 and 3 were kept as normals while dogs 4, 5 and 6 were totally depancreatized. Eighteen hours after a meal a sample of blood was drawn, followed by an intravenous injection of 10 to 25 mgm. of purified secretin. This represented from ten to twenty-five times the secretagogue dose of the preparations. Subsequent samples of blood were drawn at 30 minutes, one hour and one and one-half hours.

Table 1 shows the protocols from one of the normal animals and one of

the diabetic animals. These are not selected cases but are typical examples.

DISCUSSION. These results seem rather conclusive in showing that purified secretin does not act on the blood sugar either in the normal or diabetic dog. No doubt the hypoglycemic properties reported by others have been due to impurities contained in the secretin. Mellanby suggests

TABLE 1

Showing the effect of secretin on the blood sugar of normal and diabetic dogs

NORMAL									DIABETIC								
Day	Weight	Dose of secretin	Blood sugar				Urine volume	Urine sugar	Day	Weight	Dose of secretin	Blood sugar				Urine sugar	Urine volume
			Before	30 min.	1 hour	1½ hours						Before	30 min.	1 hour	1½ hours		
	kg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*cc.*	*gm.*		*kg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*gm.*	*cc.*
1	15.8	0	82				325		1	16.2	0	93					360
2	15.7	0	81				475		2	16.1	0	87					180
3	15.6	0	89				360		3	16.3	0	90					420
4	15.9	5	84	87	82	88	270		operated								
5	15.0	0	86				290		4	16.3	5	180	195	175	167		180
6	14.8	10	90	84	92	80	300		5	16.1	5	220	215	210	210	0.9	90
7	15.0	0	81				315		6	16.2	5	265	265	270	273	1.7	270
8	14.5	10	93	90	96	94	225		7	16.1	0	225				2.3	360
9	14.9	10	90	84	86	80	290		8	15.8	10	272	263	275	274	2.9	480
10	14.0	0	90				200		9	15.3	10	260	261	263	270	3.2	520
11	14.2	10	91	95	92	92	360		10	15.0	10	253	261	259	259	4.1	730
12	14.4	0	80				470		11	15.0	0	260				3.6	640
13	14.7	25	84	90	86	94	280		12	14.8	10	280	281	285	280	2.4	395
14	14.6	0	87				270		13	14.8	10	259	261	263	263	3.3	570
15	14.8	25	81	87	82	80	225		14	14.7	0	275				3.2	560
16	14.8	25	84	81	86	80	280		15	14.2	0	290				4.2	735
17	14.9	0	82	84	80	80	300		16	14.1	0	310				4.0	720
									17	14.0	25	243	249	246	241	4.7	840
									18	14.3	25	247	240	231	231	5.1	860
									19	14.2	25	253	259	261	270	3.9	700
									20	14.6	0	240				2.0	320
									21	14.1	10	220	227	231	223	3.0	480
									22	14.0	25	215	220	220	220	2.4	360

that the impurities are insulin. He reported that one milligram doses of his secretin injected subcutaneously in the cat did not affect the blood sugar. On the other hand, Zunz and LaBarre found that secretagogue doses (1 to 4 mgm.) of his product injected intravenously in the dog caused a marked decrease in the blood sugar. The secretin used in these experiments in doses as high as 2 mgm. per kilo did not cause alteration of the blood sugar.

CONCLUSIONS

1. Purified preparations of secretin injected intravenously in normal or diabetic dogs, barbitalized or unanesthetized, do not alter the blood sugar.
2. Secretin per se does not have insulin-like properties.
3. Secretin does not stimulate the secretion of insulin from the pancreas.

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CHANGES IN VOLUME OF THE SPLEEN IN RESPONSE TO ACOUSTIC STIMULATION

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During the course of observations on the changes in the volume of the spleen, Hargis and Mann noted that the organ showed definite responses to ordinary noises that occurred in and around the laboratory during working hours. The sudden entrance of some one into the laboratory, the slamming of a distant door, and the like, caused sudden and marked splenic contractions.

Additional observations on the responses to external stimuli have been made in the course of our experimental work on acoustic-visceral reflexes. An attempt has been made to study the nature and conditions of these responses. The same stimuli were used as in the previously reported experiments (1), that is, oscillator sounds of known wave form, frequency and duration, with constant intensities that can be repeated. The usual frequencies employed were the octaves of C: 128, 256, 1024 and 2048 vibrations each second. A number of observations were also made using frequencies of 6500 and 8000 vibrations each second. The intensities have been kept fairly constant throughout and well within the range of human audibility, but sufficiently moderated in intensity so that fear-like reactions were not produced apparently in the experimental animals. The special sensitivities of the different animals necessitated certain adjustments in the loudness of acoustic stimuli but never to an extreme degree in either direction. With the exception of one experiment in which a cat was used, dogs were used in all of the experiments.

EXPERIMENTAL PROCEDURES. The collodion plethysmographs described by Hargis were used. Several plethysmographs of different sizes were made and sterilized before each operation so that a proper selection could be made according to the size of the organ.

The abdomen was opened surgically under ether anesthesia and the spleen covered with omentum. The plethysmograph was then adjusted, sutured and sealed with strips of gauze dipped in collodion. The remainder of the omentum was reflected over the outside of the plethysmograph and sutured lightly. A stab wound was made in the abdominal wall and the

tube of the plethysmograph was brought out through it in such a manner that the organ remained in as normal a position as possible. The abdomen was then closed and the wound covered with a strip of gauze and collodion. Each animal was given from three days to a week to recover from the operation before any observations were made.

Before and after the operations the animals were brought to the laboratory, made familiar with their surroundings, trained to lie quietly on the table and allowed to hear the stimuli which were later used in the tests. With this preliminary training, they were easily handled when the actual observations were being made. Extraneous disturbances were avoided as much as possible. For this reason, many of the experiments were done during the night.

When the records were being taken, the animal was permitted to lie on its side on a comfortably padded table. Mechanical restraint was not necessary. The tube leading from the plethysmograph was attached by means of a glass cannula and a rubber tube which led to a water manometer. At times it was necessary to aspirate the plethysmograph before the experiment was begun in order to remove the fluid exudate which had collected. Simultaneous respiratory tracings were made in many of the experiments.

A signal magnet introduced into the circuit of the oscillator showed the period of stimulation on all of the records. The animal was always so placed that it could not see the movements of the observer on turning on the source of the auditory stimuli; this was done either by a foot switch or by a mercury dip hand switch.

The records were made on smoked kymograph drums with a sliding writing point which followed the meniscus of the water manometer.

In all instances the animals were allowed to settle down for a period of ten to thirty minutes before observations were begun. Then a preliminary test was made at the beginning of each record to find the normal fluctuations in the volume of the organ before the stimuli were given.

Results. From the experimental evidence furnished by these investigations, it may be said that acoustic stimulation usually causes contraction of the spleen. There are a few exceptions to this general rule and reference will be made to them.

The observed responses may be divided into a number of types: 1, sudden contraction with the onset of the stimuli; 2, delayed sudden contraction occurring during the application of the stimuli; 3, sudden contraction following the cessation of the stimuli; 4, slow contraction during the application of the stimuli; 5, persistent contraction, and 6, dilatation during the application of or following the stimuli.

The type of response which occurred with a given stimulus was apparently conditioned by preceding responses. Nothing can as yet be said

concerning the relative effectiveness of the various pitches in producing the reactions. Three factors, however, have been shown to determine the type of response. These are: 1, the period of duration of the stimulating sound (whether prolonged, short or repeated); 2, the number of repetitions of the same stimulus, and 3, change of pitch or the introduction of a new type of stimulus.

Sudden contraction with the onset of the stimuli has usually been found associated with the first stimulus in an experiment or with a stimulus given after a considerable period of rest (fig. 1). It also may accompany a change of pitch when the preceding stimuli no longer produced any effect.

Delayed sudden contraction occurring during the stimuli, a number of seconds after their onset, was noted. It was associated with prolonged stimulation after the same frequency of acoustic vibration had been repeated a number of times. The contractions usually occurred from fifteen to thirty seconds after the onset of the stimuli (fig. 2).

Sudden contraction following the cessation of the stimuli was not very frequent, but when it did occur it followed acoustic stimuli which had been repeated a number of times without producing either immediate or delayed contraction (fig. 3).

Slow contraction during acoustic stimuli occurred in two conditions: when the form of the stimulus had been changed to a series of short sounds after the same frequency of vibration had been applied as a number of prolonged stimuli (fig. 1), or during prolonged stimuli after the same frequency of vibration had been applied as a number of short, single stimuli which, in themselves, did not produce any effect (fig. 4).

Persistent contractions were observed a number of times. They were associated with a series of either long or short stimuli of the same frequency. The spleen was found to remain contracted for fifteen to thirty minutes or longer. In one instance, the dog became restless some time after the development of the prolonged contraction, so that continuous observation had to be abandoned. However, several hours later, the same animal failed to show any of the typical splenic responses which had been manifested earlier in the day. Two examples of persistent contractions are represented in figure 5, *a* and *b*, and in figure 6. The tracing shown in figure 5 was obtained immediately after the one shown in figure 1.

Dilatation was not frequently observed. Occasionally the spleen was shown to dilate after a short stimulus which had been repeated a number of times without producing any previous effect. Sometimes an extrinsic disturbance would be accompanied by rapid, marked dilatation (fig. 7).

The spleen of the one cat in this series presented uniform, normal curves, with regular sinusoidal waves every twenty-five seconds on which were

superimposed smaller fluctuations corresponding to the heart beats and respirations. With acoustic stimuli given by the oscillator, marked changes were not noted in the splenic curve. Only a flattening out of the larger waves was associated with these sounds. However, when a mechanical sound was made, as by the whirling of a tin rattle, the normal

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Fig. 1. Contraction of the spleen at the onset of acoustic stimulation, and during a series of rapidly repeated stimuli. The frequency of the stimuli was 256, and the duration fifteen, thirty-five and fifty-five seconds, respectively, the last being a series of rapidly repeated sounds. The normal splenic contraction waves are suddenly interrupted at the beginning of the first stimulus. There is only a slight change in volume during the second stimulus, and a slow but marked contraction occurs during the repeated sounds.

Fig. 2. Delayed but sudden contraction of the spleen occurring twenty-two seconds after the beginning of an acoustic stimulus of 256 vibrations a second lasting forty-four seconds. This stimulus was preceded by a number of stimuli at the same frequency. The time marker was set at five seconds.

Fig. 3. Contraction of the spleen after the cessation of a stimulating sound having a frequency of 256 vibrations a second, lasting seventeen seconds. This stimulus had been preceded by a number of tones of higher pitches which were not accompanied by any changes in the volume of the spleen. Upper tracing, respiration. The time marker was set at one second.

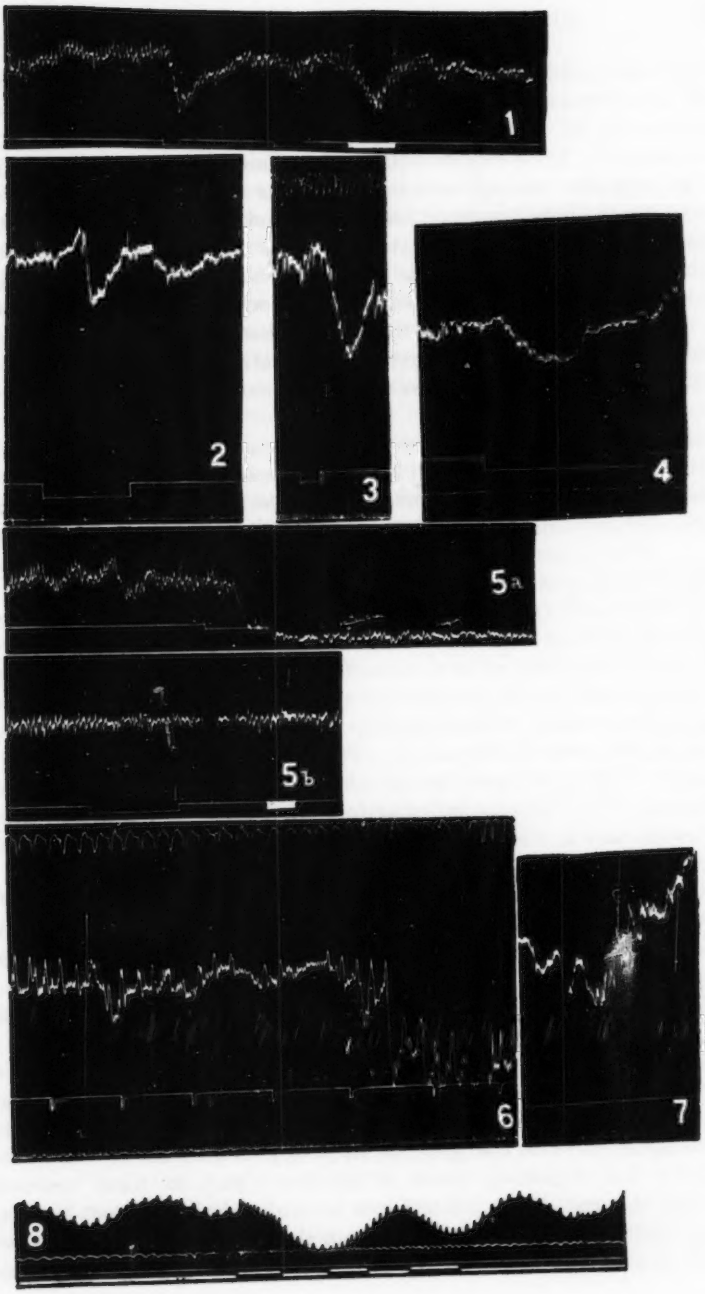
Fig. 4. Slow contraction of the spleen during an acoustic stimulus of 800 vibrations a second, lasting one minute. The preceding stimuli, short sounds at the same frequency, had been without effect.

Fig. 5. Persistent contraction of the spleen commencing during an acoustic stimulation of 256 vibrations each second and lasting two and a half minutes. Fluid was added to the manometer between *a* and *b* to bring the tracing to a convenient level. The first stimulus used in *b* was a single sound of 256 vibrations a second; the second stimulus was a series of short sounds at 2048 vibrations a second. These were without effect on the volume of the spleen. Toward the end of *a* the dog was apparently sleeping. He opened his eyes again before *b* was begun, but other visible movements were not made.

Fig. 6. The beginning of a persistent contraction of the spleen during a series of short stimuli given every thirty seconds. The portion of the tracing shown begins at the fifteenth stimulus. At the sixteenth stimulus there is a slight contraction with a rapid return to the normal level. After the nineteenth stimulus the respiratory fluctuations exhibited by the spleen suddenly increase in amplitude without any visible disturbance in the respiratory tracing. The spleen became contracted markedly and was observed continuously for half an hour thereafter, during which period change did not occur in the level of the splenic volume. The same stimuli, repeated twenty minutes after the beginning of the contraction, were without effect. The upper tracing represents respiration.

Fig. 7. Dilatation of the spleen during extraneous disturbance; some one entered the adjoining room whistling. The dog did not appear to move.

Fig. 8. Effects on splenic volume in a cat produced by a sound made by whirling a tin rattle. Three similar sounds were given, each lasting about ten seconds. The upper line represents changes in splenic volume; the second line, respirations; third line, time in seconds, and the fourth line, signal. (Photographic method, subsequently used in connection with changes in volume of kidney.)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waves were suddenly interrupted, the spleen contracted and the respiratory waves increased in rate. Additional stimuli failed to produce any reaction (fig. 8).

COMMENT. These experiments seem to show that external stimulation of an organism through acoustic vibrations causes marked visceral responses in the form of splenic contractions and dilatations. The fact that these changes have been so extensive under ideal laboratory conditions seems particularly significant. The experimental animals were made thoroughly familiar with their surroundings, with their observers and with the stimulating sounds before the tests were made. When the responses occurred, the animals did not give any external evidence of being disturbed. This is shown in some of the records by the smoothness or regularity of the respiratory tracings.

Many of the phenomena of reflex activity, as described by Sherrington in the spinal preparation (dog), have been closely paralleled by the splenic responses. Although the peculiarities of the conduction path of these reactions have not been fully investigated, many characteristics comparable to those of general reflex activity are undoubtedly exhibited. Sherrington emphasized the fact that even in the cord, below the level of transection, each type of reflex elicited has its own idiosyncracies depending on the path and the number and distribution of the neurons involved. The acoustic-visceral reflex is exceedingly complex at its point of origin. As was pointed out in another paper (1), there are three points in the brain-stem at which connections may be established between the auditory fibers and the reticular formation. These connections are: 1, the level of entrance of the eighth nerve in the medulla through certain root or secondary fibers; 2, the pontine reticular formation associated with the lateral lemniscus, and 3, the pontine and bulbar reticular formation associated with collateral paths from the descending tectobulbar tract. Which of these paths is active in the splenic reflex has not been determined. The possibility exists that all of them are involved to some extent. Moreover, the extensive and diffuse region in the brain-stem which is known to exert vasomotor influences has not been analyzed into locally active divisions. It may be that local vasomotor effects are determined through the mediation of cells of the intermediolateral column of the spinal cord.

However, in the face of this anatomic complexity, some features of reflex activity certainly are manifested by the acoustic-splenic responses. The extinction of the end effect on repetition of the same stimulus, followed by its rapid return on changing the frequency or the form of stimulation, suggests that inhibition plays a significant part in these reactions. Whether the seat of this inhibition is cortical or bulbar cannot be ascertained from data at hand, since the animals used in the experiments possessed intact central nervous systems. It may be that fatigue plays some

part in the disappearance of the end effect, but the rapidity and completeness of this disappearance makes it more likely that the process is one of inhibition.

In the delayed reactions which have been so frequently observed, there is evidence either of a protracted refractory phase or a phenomenon of summation. Interpretation must be deferred until more is known of the electric response of the auditory fibers. Whether there is a continuous phasic response of this nerve corresponding to the frequency of the stimulus, or whether the stimulus acts simply in the manner of a make and break induction shock remains to be determined. By analogy with the electric phenomena occurring in the optic nerve, the latter interpretation would seem to be the more correct. The fact that release effects have been met so frequently in these and previously reported investigations also supports such a view. Whichever of these possibilities may be found to be representative of the truth, the frequency of the occurrence of delayed splenic responses in the absence of skeletal disturbance in the animals certainly establishes this phenomenon as a definite characteristic of this particular reflex arc.

The persistent contractions which have been described as occurring after repeated stimulation of the same frequency undoubtedly represent the effects of summation of stimuli. That a series of stimuli may evoke, in the course of their application, responses of entirely different nature confirms the suggestion made previously that several of the paths between the auditory fibers and the reticular cells may be involved in the reactions. It is quite possible that one path, active in immediate responses, may become quickly inhibited or fatigued, whereas another path, involving a greater number of neurons, may become patent only after the summation of a certain number of stimuli, inadequate for eliciting rapid responses.

The changes in splenic volume, although usually in the nature of contractions, were exhibited as marked dilatations under certain conditions. Thus we have evidence of opposing mechanisms in the functioning of which another condition of reflex activity is present, namely, reciprocal inhibition. Whether this can ever be as conclusively demonstrated as in the case of spinal reflexes is doubtful. One can judge only from the evidence of the experimental results. The spleen is able to respond to acoustic stimulation either by contraction or by dilatation. When one mechanism is active, the other must be inhibited. That this second mechanism is not merely inactive but actually inhibited seems evident from a consideration of the diffuse spread of acoustic impulses in the brain-stem. It does not seem likely that a selective activity between auditory fibers and vasoconstrictors, on the one hand, and auditory fibers and vasodilators, on the other hand, could ensue on adequate stimulation. That activation of both sets takes place simultaneously, manifesting itself as an excitatory

phenomenon in the one set of fibers and as an inhibitory phenomenon in the other set, is an interpretation which conforms with the general rules of reflex action.

SUMMARY

Experiments have been performed on dogs and on one cat in which the changes in volume of the spleen in response to acoustic stimulation have been graphically recorded. These changes were studied by the plethysmographic method described by Hargis. The environmental conditions of the experimental animals were carefully controlled. External evidences of emotional response were not noted at any time.

The splenic responses were found to follow certain definite types. These are: sudden contractions with the onset of the stimuli, delayed sudden contractions occurring during the stimuli, sudden contractions following the cessation of the stimuli, slow contractions during the stimuli, and persistent contractions and dilatations. The conditions under which these various responses were obtained are briefly described and a number of tracings illustrating these responses are reproduced. It was pointed out that these reactions have some of the characteristics of general reflexes which were described by Sherrington.

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CHANGES IN VOLUME OF THE KIDNEY IN RESPONSE TO ACOUSTIC STIMULATION[1]

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A series of observations was made on the renal responses to acoustic stimulation following the same procedure as that adopted in the experiments on the changes in splenic volume (3). Reid has recently described the method with special reference to the kidney. The left kidney was enclosed, as in the case of the spleen, in a collodion plethysmograph of the type described by Hargis. The dogs which were used throughout the experiments were trained a number of days before the experiments were performed. Both the spleen and the kidney in one animal were enclosed in plethysmographs and simultaneous records made from both organs.

The records were made by photographing (1) the moving meniscus of a water manometer and the shadows cast by the levers (indicating respiration, time, and the stimulation signal) with a motor-driven revolving camera. The kidney was connected with the manometer and the spleen with a Marey tambour when both organs were observed simultaneously.

The same stimuli were used as in the experiments on the spleen (3), that is, tones produced by means of an electric oscillator. In general the frequencies used were the octaves of C up to 2048 vibrations each second, and occasionally frequencies of 6500 and 8000 vibrations each second.

EXPERIMENTAL RESULTS. For convenience in designating volumetric changes in the kidney, a decrease in volume will be spoken of as a contraction, and an increase as a dilatation. These terms will therefore indicate simply the total changes as recorded by the plethysmograph, whether they are entirely vasomotor in nature, or whether some secretory factor also contributes to the final effect.

As in the case of the spleen, the responses of the kidney associated with acoustic stimulation followed certain definite types. These were: 1, sudden contraction with the onset of the stimuli; 2, slow contraction during the application of the stimuli; 3, sudden contraction at the cessation of the stimuli; 4, slow contraction at the cessation of the stimuli; 5, delayed contraction, and 6, dilatation.

[1] Work done in the Divisions of Physics and Biophysical Research and of Experimental Surgery and Pathology.

The splenic contractions of the dog in which simultaneous observations of both organs were made followed the general types outlined in a preceding paper (2). They will be referred to again in connection with the renal changes.

The experiments in this series were all performed at night in order to minimize as much as possible extraneous sources of stimulation. The responses were typical only so long as other sounds were absent. As soon as daybreak brought the twittering of birds the visceral responses became irregular and no longer followed any of the established types, although the trained animals did not make the slightest movement.

Fig. 1. Contractions of the kidney in response to acoustic stimulation. The first contraction is an immediate response at the beginning of stimulation. The second contraction is a delayed reaction, occurring three seconds after the second stimulus. The stimuli were a series of short sounds at 2048 vibrations a second given every five seconds. Upper line, changes in kidney volume; second line, respiration; third line, time in seconds; fourth line, signal indicating acoustic stimuli. This description will apply to subsequent figures, unless otherwise stated.

Fig. 2. Slow contraction of the kidney during acoustic stimulation. There is a slight increase in volume following the initial contraction, after which gradual contraction begins, and persists throughout the period of stimulation. At the cessation of the stimulus the volume returns to normal. The stimulus was a sound 6500 vibrations a second lasting thirty seconds.

Fig. 3. Slow contraction of the kidney at the cessation of acoustic stimulation. The stimuli were single short sounds at 6500 vibrations a second given every five seconds for three minutes. The kidney had recovered almost completely from contractions which had occurred during the early part of the period of stimulation. The renal disturbance began to manifest itself five seconds after the last stimulus had been given and at the moment that the next stimulus might have been expected.

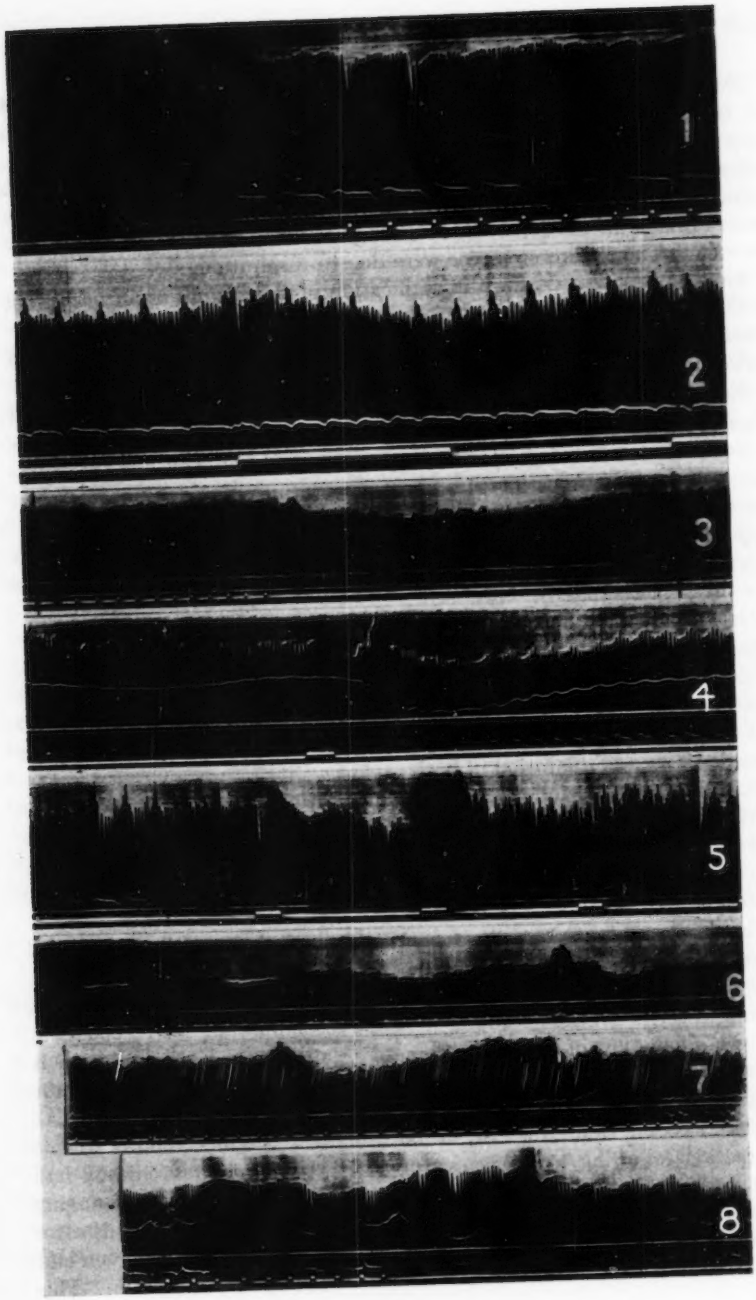
Fig. 4. Delayed contraction of kidney and spleen following acoustic stimulation. The stimulus consisted of two sounds of 700 and 1000 vibrations a second respectively. The contraction was preceded by a rapid dilatation of both organs. The second line represents changes in splenic volume. Description is otherwise as that in figure 1.

Fig. 5. Dilatations of the kidney following sudden sharp contractions. The stimuli were sounds of 256 vibrations each second and lasting five seconds each.

Fig. 6. Dilatations of the kidney with simultaneous contractions of the spleen during a group of short sounds of 2048 vibrations each second. The pitch of the first group of stimuli was 1000 and of the second 256. A reaction did not occur until the third group of stimuli was applied with a frequency of 2048. The second line shows changes in splenic volume.

Fig. 7. Opposite reactions of the kidney and spleen during a series of acoustic stimuli at a frequency of 2048 repeated every five seconds for five minutes. The second line shows changes in splenic volume.

Fig. 8. Splenic contractions after a series of single short stimuli repeated every five seconds for fifteen minutes. The frequency of the stimuli was 256 for the first and the last five minutes, and 2048 for the second five minutes. Volume changes were not noted either at the beginning or during the course of the period of stimulation, but exactly five seconds after the last sound, when the next stimulus might have been expected, the spleen contracted. This tracing was made at the end of a long experiment of several hours' duration. The second line shows changes in splenic volume.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Sudden contraction with the onset of the stimuli was the most frequent of the renal responses. It usually accompanied the initial stimulus in any given experiment or occurred following a stimulus given after a considerable period of rest. It was observed also when the pitch of the stimulating sounds was changed. Figure 1 represents the changes in renal volume. Sharp fluctuations, which correspond with the cardiac beats, may be seen. The shallower waves were due to respiration.

Slow contraction during the application of the stimuli occurred during prolonged sounds, sometimes after recovery from an initial sudden contraction (fig. 2). The contractions have been observed also during a series of repeated short sounds with which an initial effect was not observed.

Sudden contraction at the cessation of the stimuli has been observed after a series of stimuli, either of constant or varied frequencies, which were ineffective in bringing about any concomitant effects. In the examples of this type of reaction the curve representing splenic responses, when present, closely paralleled the curve representing renal responses.

Slow contraction at the cessation of the stimuli generally followed a long series of short sounds during which recovery from the initial effects had taken place (fig. 3).

Delayed contraction occurred frequently and usually from three to fifteen seconds after the cessation of the stimuli (fig. 1). The spleen showed, at times, a response which was parallel to that of the kidney (fig. 4) and at other times divergent. The stimulus consisted of two sounds of 700 and 1000 vibrations each second, respectively. The contraction was preceded by rapid dilatation of both organs.

Dilatation usually followed sudden contractions (fig. 5). It seemed to be most definite if the initial response was absent or weak. Dilatations may also be associated with changes of pitch. The marked renal dilatation shown in figure 6 is accompanied by an equally marked splenic contraction.

Figures 7 and 8 are records of reactions which do not follow the usual types. Figure 7 shows the divergence in renal and splenic responses which sometimes occurs. These reactions took place during the application of a prolonged series of short stimuli. The curves show that the responses of the two organs are of opposite character, that is, a splenic dilatation is accompanied by a renal contraction. After recovery the renal volume remains stationary while the spleen dilates. A short time later a small dilatation occurs simultaneously in both organs. Figure 8 shows splenic contraction following a long series of short stimuli. These stimuli had been given at intervals of five seconds for fifteen minutes without bringing about any changes of volume in either organ. Exactly five seconds after the last sound, at which time the next stimulus would have been given had the series been continued, the spleen showed a sudden contraction. This

may be termed an "expectant" reaction. Similar responses have been observed on a number of occasions.

COMMENT. The responses of the kidney to acoustic stimulation have been shown to follow types that are dependent on the mode of stimulation. In a preceding paper, dealing with responses of the spleen to similar stimuli, the reactions were considered from the standpoint of certain relationships between them and the spinal reflexes studied by Sherrington. In the case of the kidney, also, there is another group of reflexes to which this comparison is again applicable. Simultaneous graphs of the changes in the renal and splenic volumes show that the reactions of the two organs are not necessarily similar in direction, but that the responses may occur independently or in opposite directions. The two organs have independent vasomotor innervations, as has been established by Bradford and Gaskell.

The question arises as to the point of origin of the different reactions. Is it in the reticular formation of the brain-stem or in the spinal cells giving rise to the sympathetic neurons? That the problem is an extremely complex one becomes evident with the accumulation of data. Evidence is present of changes in respiration, heart rate, general blood pressure and in splenic and renal volumes. Just how much one effect influences another cannot as yet be estimated. It is certain that they all occur simultaneously and it is also fairly certain that the path of each reflex begins somewhere in the reticular formation of the medulla and pons. Does this complex nervous network contain separate conduction paths, or are the cells so interrelated that they have no functional individuality but react entirely according to existing local conditions? The fact that the responses occur again and again and are of like character under similar conditions of stimulation undoubtedly points to a certain degree of organization within the reticular formation. Whether this will lend itself to anatomic analysis is a problem for further investigation.

SUMMARY

Graphic tracings of the changes in volume of the kidney in dogs in response to acoustic stimulation are presented. The method and the experimental conditions were the same as in the previously reported experiments on the spleen (3). Simultaneous tracings were made of the responses of the kidney and the spleen to acoustic stimulation.

The responses of the kidney followed definite types dependent on the mode of stimulation. These types of responses are: sudden contraction with the onset of the stimuli, slow contractions during the application of the stimuli, sudden contractions at the cessation of the stimuli, slow contractions after the cessation of the stimuli and delayed contractions and dilatations. An account of the conditions under which these various reac-

tions occur is given in the text together with a number of illustrative tracings.

In the animals in which simultaneous observations were made of both the kidney and spleen it is noted that the responses do not always parallel each other, but occur sometimes independently in one organ or the other, sometimes in the same direction in both organs and sometimes simultaneously but in opposite directions.

The anatomic possibilities for the origin and paths of the visceral acoustic reflexes are discussed briefly.

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STUDIES IN THE PHYSIOLOGY OF VITAMINS

VIII. The Effect of Parathormone on Normal and Vitamn B-Deficient Rats[1]

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Part I. Testing parathormone for the possible presence of vitamin B.[3] Parathyroid tetany and vitamin B deficiency in dogs have certain features in common. These include diminished food and water intake, vomiting, neuro-muscular disturbances, and lowered resistance to infection. Furthermore, Carlson (1916) has described the occurrence of gastric atony in parathyroid tetany and it has recently been demonstrated (Rose, Stucky and Cowgill, 1929) that some dogs suffering from B-avitaminosis manifest atony of the stomach. More striking was the report of Smith (1927) that the blood calcium of dogs exhibiting symptoms of "beriberi" was near the tetany level. These considerations, together with the fact that the authors were investigating the effect of parathormone on gastric motility in vitamin B-deficient dogs, rendered it highly desirable to test this hormone preparation for the possible presence of the complex known as vitamin B.

Experimental details. The parathormone[4] was prepared for the feeding trials by mixing 1100 units of the clear extract with 5.5 grams of pure dextrin and gently evaporating to dryness below 45°C. at which temperature the vitamin is perfectly stable (Osborne and Mendel, 1918; Sherman and Burton, 1926). When completely dried, the mass was thoroughly ground and yielded a fine white powder. Approximately 130 mgm. of this parathormone-dextrin preparation were equivalent to 25 units of the hormone.

[1] The data in this paper are taken from a dissertation submitted by William B. Rose in partial fulfilment of the requirement for the degree of Doctor of Philosophy, Yale University, 1928.

[2] Porter Fellow of the American Physiological Society, 1926–27.

[3] The term vitamin B, as used in this paper, refers to the combination of two or more factors which have been shown to make up this substance (Smith, 1928).

[4] A potent extract of the parathyroid glands obtained through the courtesy of Eli Lilly & Company, Indianapolis, Indiana.

The tests for vitamin B were made on a group of twelve albino rats from the same litter, 26 days old, and bred in this laboratory. Each animal was housed separately. Cylindrical, wire-mesh cages with raised screen floors were utilized to prevent coprophagy (Smith, Cowgill and Croll, 1925).

During the first two weeks of the experiment, all the rats were fed a basal vitamin B-deficient ration (table 1) supplemented by 200 mgm. of dried brewery yeast[5] per rat per day. At the end of this period 3 of the animals were continued on this dietary régime as controls. The remaining 9 rats were deprived of the vitamin B supplement (yeast) for seven days, at which time growth had ceased. Six of these deficient rats were then given daily supplements of 130 mgm. of the parathormone-dextrin preparation (equivalent to 25 units of the hormone) for seven days, when 400 mgm.

TABLE 1

Vitamin B deficient ration

	PER CENT
Meat residue*	18
Corn starch	50
"Crisco"	23
Cod liver oil	5
Salt mixture**	4

* This substance is practically free from vitamin B (G. R. Cowgill. 1926. THIS JOURNAL, lxxix, 341). It may be obtained from the Valentine Meat Juice Company, Richmond, Va.

** The salt mixture described by Osborne and Mendel (Journ. Biol. Chem., 1917, xxxii, 374) was employed.

of yeast per animal daily were substituted for the hormone preparation. The other 3 vitamin B-deficient rats were each given a daily subcutaneous injection of parathormone (25 units). This procedure was selected for the purpose of duplicating as far as possible the physiologic effect of the parenteral administration of this hormone to dogs in order that the results of the studies on gastric motility, following parathormone injection into vitamin B-deficient dogs, could be better interpreted.

EXPERIMENTAL RESULTS. The three control rats continued to gain in weight and showed growth even better than that cited as normal by Donaldson. In contrast to the latter all of the six vitamin B deficient animals, receiving the parathormone-dextrin preparation, continued to lose in weight and developed the usual symptoms of B-avitaminosis. Within a few days after yeast therapy was instituted, the food intake increased

[5] Obtained from the Northwestern Yeast Company, Chicago.

considerably and abnormally rapid growth ensued. The experiment was terminated on the eleventh day of the realimentation period.

The three animals given daily parenteral injections of the hormone (25 units) declined progressively in body weight. Moreover, they became very quiet and all three were found dead on the morning following the third injection.

Discussion. The following considerations prompted the writers to use relatively large doses of the hormone. Firstly, if the endocrine substance contained very small quantities of vitamin B, the latter would be more readily detected by the biologic technique employed. Secondly, the possibility of incomplete intestinal absorption in the case of vitamin B deficient rats was entertained. Thirdly, because the basal metabolic rate is so much greater in the rat than in other relatively large experimental animals, a proportionately larger dose was deemed advisable.

It is obvious that the vitamin B-deficient ration, supplemented by yeast daily, was adequate for normal growth of these animals. The substitution of the parathormone-dextrin preparation for yeast failed to furnish an adequate source of vitamin B as indicated by the progressive loss in body-weight. In view of the relatively large quantity of the hormone incorporated in the dextrin preparation (over three hundred units per kilo for each rat daily) it is apparent that parathormone does not contain any appreciable amount of vitamin B or is practically devoid of it, as far as one can judge from these experiments.

The fatal results obtained in those rats which were given repeated injections of the endocrine extract deserves special consideration. Collip (1926) reported that this species is apparently immune to the effect of the potent chemical substance which he obtained from the parathyroid glands. For this reason, the authors were somewhat surprised to find that the animals died after the third injection of the hormone.

Until an adequate explanation for the fatal results obtained was forthcoming, it was thought best not to hazard an interpretation of these data. Furthermore, it was essential for us to investigate this question before we could feel justified in proceeding with our experiments on the effect of parathormone on gastric motility in dogs, because the same endocrine preparation was employed in both experiments.

Part II. The effect of parathormone on normal rats. The problem which confronted us was to attempt to determine the cause of death in rats given subcutaneous injections of parathormone—a hormone toward which this species was believed to be immune. A number of possibilities was entertained at this time. First, the age of the rat may be a factor influencing the susceptibility to this organ extract. Secondly, the hormone may have been contaminated with some highly toxic substance. Since parathormone is widely recommended for clinical

use, the importance of settling this question needs no emphasis. Thirdly, rats may not be immune to massive doses of the hormone. Fourthly, vitamin B deficient rats may be peculiarly sensitive to this product. Finally, before all possible extraneous factors could be eliminated, it was essential to confirm these results on other rats, if the cause of death were to be attributed solely to the hormone injections. Accordingly, the following experiments were performed.

Experiment 1. Each of three rats, weighing 128, 121 and 114 grams respectively, was given daily subcutaneous injections of thirty units of parathormone. All three rats died on the day following the third injection. At autopsy, serous exudate in the chest and marked dilatation of the stomach were found. These results indicate that repeated subcutaneous injections of this sample of the hormone to apparently normal rats are attended with fatal results.[6]

Experiment 2. Each of four young rats (age, 23 days), weighing 40, 42, 45 and 50 grams respectively, was given daily subcutaneous injections of the same hormone. Each animal received approximately thirteen units daily (the dose being the same per unit weight as that given to the other rats). These animals survived four successive injections.

Inasmuch as these rats survived, it would appear that younger rats are less susceptible to the effects of this endocrine extract. Furthermore, it is highly improbable that the hormone preparation used in this investigation contained an extraneous toxic substance. It is of interest to note that young animals are more susceptible to parathyroid deficiency than adults (Horsley, 1886; MacCallum, Thomson and Murphy, 1907). The removal of only three of the parathyroid glands may cause fatal tetany in a young dog, whereas, it is usually without such an effect in aged animals. It has also been demonstrated by Dragstedt and Sudan (1926) that proportionally more calcium lactate is required to control tetany after thyro-parathyroidectomy in young than in adult dogs. Old animals may fail to show any symptoms after thyro-parathyroidectomy (Simpson, 1924).

Experiment 3. It then became desirable to investigate further into the cause of death in these older rats that died after parathormone injection. Inasmuch as it has been frequently reported that parathormone produces a persistent hypercalcemia in dogs and that this may prove fatal, it was natural to inquire whether or not this same factor obtained in the case of rats in spite of the fact that it has been reported that rats are immune.

Consequently, seven apparently normal, male rats ranging from 245 to 314 grams in weight were given subcutaneous injections of this hormone

[6] While this paper was being prepared for publication, similar results were reported by Tweedy and Chandler (THIS JOURNAL, lxxxviii, no. 4, May, 1929, p. 754).

employing approximately the same dose as before (25 units per 100 grams of body weight per day). Twelve hours after the third injection four of the rats were moribund, and the rest looked very ill. Each rat was anesthetized with ether and the left fore paw amputated. The blood was collected and the serum calcium determined by the Clark-Collip (1925) modification

TABLE 2

Blood serum calcium values of normal rats after subcutaneous injections of parathormone

SAMPLE NUMBER	SOURCE OF BLOOD	BLOOD SERUM CALCIUM
		mgm. per 100 cc.
1	Pooled blood (2 rats)	14.0
2	Pooled blood (2 rats)	16.3
3	Blood from one rat	16.1
4	Blood from one rat	13.1
5*	Pooled blood (7 rats)	14.7
Average		14.8

* The blood for this sample was obtained chiefly from the seventh rat together with small quantities left over from the other 4 samples. Enough blood serum was thus obtained for an additional estimation.

TABLE 3

Blood serum calcium values of normal rats

SAMPLE NUMBER	SOURCE OF BLOOD	BLOOD SERUM CALCIUM
		mgm. per 100 cc.
1	Blood from one rat	10.7
2	Blood from one rat	10.1
3	Blood from one rat	10.8
4	Pooled blood (2 rats)	10.3
4*	(Duplicate estimation)	10.1
5	Pooled blood (2 rats)	10.0
6	Pooled blood (2 rats)	10.5
7	Pooled blood (2 rats)	10.9
7*	(Duplicate estimation)	10.5
Average of the seven samples		10.4

* By pooling the blood from 2 rats, enough serum was obtained to run duplicate estimations.

of the Kramer-Tisdall (1921) method. The results of the blood calcium determinations of these rats are given in table 2.

As a control on the technique employed in these determinations, blood samples were obtained from eleven apparently normal rats. These animals varied in size, age, weight and sex. The blood calcium values are given in table 3.

The data obtained for the normal blood serum calcium in this species indicate that the values are similar to those obtained in higher animals. This confirms the recent report of Cameron and Williamson (1927). From a comparison of tables 2 and 3, it is seen that the average blood serum calcium of albino rats after subcutaneous injections of parathormone is 4.4 mgm. higher than the average value obtained from normal rats. This represents an increase of 42.3 per cent.[7]

From the work of Underhill (1920) and others, it is clear that a sudden and pronounced blood concentration may produce death. The work of Collip (1925) indicates that a definite increase in blood concentration may result from the parenteral administration of a potent extract of the parathyroid glands. Hence, it is highly probable that this was a contributory factor in producing the harmful effects observed in rats receiving large and repeated doses of this potent physiologic agent.

SUMMARY AND CONCLUSIONS

For reasons given above, parathormone was tested for the possible presence of vitamin B by means of a biologic assay on rats. Repeated parenteral administration of this organ extract proved fatal to vitamin B deficient rats. In view of the fact that this species has been reported to be immune to the effect of this hormone, experiments were performed to ascertain the possible cause of death.

The parathormone preparation employed does not contain any appreciable amount of vitamin B.

The rat is not immune to the effect of a potent extract of the parathyroid glands.

Repeated injections of large doses of this hormone may prove fatal to rats.

Young animals appear to be more resistant to the effects of this hormone, —inasmuch as they can withstand a dose per unit of body weight that proves fatal to older animals.

Successive injections of this glandular material produces a significant increase in blood serum calcium, which in these experiments averaged approximately 42 per cent.

The blood serum calcium of apparently normal rats of varying age, weight and sex lies within the same physiologic range as that found in higher mammals. This is in accord with the results of other investigators.

[7] In a personal interview with Professor Collip, the suggestion was made that this hypercalcemia may possibly be due, in part at least, to the anoxemia associated with the moribund state of these rats, when blood was obtained for calcium determination.

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STUDIES IN THE PHYSIOLOGY OF VITAMINS

IX. Hemoglobin, Sugar and Chloride Changes in the Blood of Vitamin B-Deficient Rats[1]

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The present communication is a report of changes in blood sugar, hemoglobin and chlorides in rats during vitamin B[3] deficiency and recovery therefrom.

The literature pertaining to the blood sugar level in B-avitaminosis is conflicting. Thus, Mattill (1923) determined the respiratory quotient of vitamin B deficient rats, and concluded from his experiments that vitamin B is not specifically related to carbohydrate metabolism. Likewise, Eggleton and Gross (1925) investigated the blood sugar level of rats fed a diet lacking vitamin B. Their results indicate nothing abnormal in the deficient animals until the stage of final collapse, when the blood sugar fell to the starvation level. Similarly, in a recent study of the blood sugar level in vitamin B deficient dogs, no significant change was found (Stucky and Rose, 1929). On the other hand a number of investigators have reported hyperglycemia in this deficiency disease (Funk and von Schönborn, 1914; Hirai, 1923; Drummond and Marrian, 1926).

It is now an established fact that when non-ruminant animals are fed a diet complete in every known factor, except with respect to vitamin B, a loss of appetite develops, sooner or later, if coprophagy is prevented. It is generally believed that starvation is accompanied by a variable increase in blood concentration (Morgulis and Edwards, 1924). In experiments on dogs (Stucky and Rose, 1929) it was found that anhydraemia of a variable degree occurs in B-avitaminosis. However, some of the control dogs also developed an increase in blood concentration. A critical

[1] Some of the data in this paper are included in a preliminary report to the Proceedings of the Society for Experimental Biology and Medicine, 1928, xxv, 687.

The data in this paper are taken from a dissertation submitted by William B. Rose in partial fulfilment of the requirement for the degree of Doctor of Philosophy, Yale University, 1928.

[2] Porter Fellow of the American Physiological Society, 1926–27.

[3] This term refers to the vitamin B complex (Smith, 1928).

study of the data suggested that the degree of anhydremia observed in both deficient and control animals appeared to be related to the percentage loss in body weight rather than to any specific effect of a lack of vitamin B. It therefore seemed highly desirable to test this viewpoint on another species—the rat.

In our earlier studies it was observed that the water intake was diminished in dogs suffering from vitamin B deficiency. Consequently, in the present investigation control rats were given on an average the same amount of water and food as that consumed by the vitamin B deficient animals. The advantage of this technique in affording a control of the possible effect of a variable food and water consumption on the relative concentration of the blood constituents studied is obvious. An examination of the technique employed by most workers in this field, revealed the fact that only *one* blood sample was obtained from each rat—and this as a rule was post-mortem blood. In the current experiments, blood specimens were obtained from both control and deficient rats at frequent intervals during the progress of vitamin B deficiency and realimentation.

GENERAL TECHNIQUE. Albino rats varying from 198 to 264 grams in body weight were employed for these experiments. One group of 14 rats was fed a basal vitamin B deficient ration (table 1) and given water ad libitum. The control group—9 in number—was started on the experiment three days later than those in the deficient group. The controls were given on an average the same amount of food and water that was consumed by the vitamin B deficient animals. In addition each control rat was given 400 mgm. of dried brewery yeast daily, regardless of its daily food allotment. The general technique developed in this laboratory for the care and feeding of the animals was followed (Smith, Cowgill and Croll, 1925). Each rat was placed in a cylindrical, wire-mesh false-bottom cage to prevent coprophagy (Steenbock, Sell and Nelson, 1923). The rats were weighed on alternate days and records of food and water consumption were kept.

In order to obtain accurate data of the water consumption of the rats, an improvised water fountain was employed. This consisted of a wide-mouthed, glass bottle (capacity, 135 cc.) fitted with a rubber stopper, carrying a glass tube bent at an appropriate angle. The bottle was attached to the cage by means of a metal collar provided with stout brass wire springs. With this arrangement, the bottle could be readily removed as required. The end of the glass tube was fire-polished and the size of the opening regulated so that no leakage occurred. A control water-bottle was kept in the rat room. However, the amount of evaporation from the tip of the glass tube was found to be negligible so that no correction factor was necessary.

Blood analyses. The diminution in blood volume that accompanies

increasing dehydration is to some extent compensated for by peripheral vasoconstriction. As a result of this, corpuscles stagnate in the peripheral vessels and a considerable difference may be observed in the cell count and hemoglobin content of blood taken from cutaneous puncture and that obtained from a large vessel or the chambers of the heart. It was therefore essential, when attempting to study blood concentration, to obtain blood from a large vessel or the heart. In the present investigation, blood was obtained by cardiac puncture in all cases.

Blood samples were taken at least once a week throughout the deficient and recovery periods. Enough blood was required for duplicate determinations of hemoglobin, sugar and chlorides. However, on account of the small size of the rat, only small amounts of blood could be safely withdrawn, if experimental anemia were to be avoided. The danger of this complication was obviated by the withdrawal of approximately 0.5 cc. of blood per rat per week.

With the small amount of blood available, it was essential to use microtechnique throughout. This necessitated the development of a micromethod for blood chlorides. A method was evolved (Rose and Stucky, 1929) which requires only 0.1 cc. of blood for a single determination. Theoretical recoveries were obtained and duplicate estimations agreed within 1 per cent. The results are expressed as mgm. NaCl per 100 cc. of blood. Blood sugar was determined by the method described by Hagedorn and Jensen (1923), which requires 0.1 cc. of blood for a single estimation. This method was found to be entirely satisfactory in previous experiments. Hemoglobin was estimated colorimetrically using a modification of the Cohen and Smith technique (1919). The acid hematin standard, made according to Van Slyke and Stadie's oxygen capacity method (1921), was checked at frequent intervals and prepared fresh as required. Hemoglobin percentages are expressed according to the Haldane scale (1900–01).

The blood was always drawn in the morning approximately three hours after the food cups had been removed from the cages. Such a sample was found by Eggleton and Gross (1925) to give the fasting blood sugar level. With the methods described above, duplicate determinations of hemoglobin, sugar and chlorides were conveniently made on approximately 0.5 cc. of whole blood.

EXPERIMENTAL RESULTS. *Anhydremia.* Evidence obtained in previous experiments on dogs (Stucky and Rose, 1929) indicated that changes in hemoglobin and total blood solids parallel each other very closely in vitamin B deficiency. Hence, in these experiments, changes in hemoglobin are used as an index of changes in blood concentration.

Figure 1 was constructed from a table of averages of the hemoglobin values of both control and deficient rats. It is apparent that there is an

initial decrease in hemoglobin during the early part of the experiment, followed by a progressive increase in this blood constituent in both control and deficient rats until realimentation was begun.

The presence of anhydremia is strikingly demonstrated in the fall of the hemoglobin values to considerably below the normal level in both groups during the first week of recovery. The average decreases in hemoglogin of the deficient and control rats during this short period were 27.1 per cent and 32.8 per cent (Haldane) respectively.

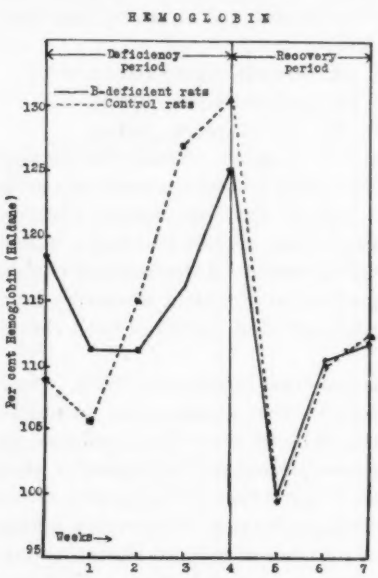

Fig. 1

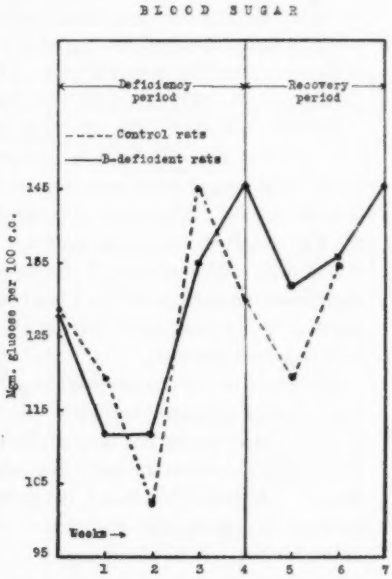

Fig. 2

The slightly greater increase in hemoglobin obtained in the control rats may be without significance. However, it should be added that these control animals were more active than the deficient rats, and this fact may possibly have been responsible in part for the difference in the two groups.

In the current experiments the decrease in hemoglobin *below* the normal level after vitamin B therapy does not necessarily indicate that any *specific* inhibitory influence on the hematopoietic system occurred during the deficient period. Recently, Sure, Kik and Walker (1929) reported that nursling young of the albino rat develop anhydremia and marked disturbance of hematopoietic function in B-avitaminosis. Their experiments are not comparable with ours. The fact that our control rats re-

ceived adequate daily supplements of vitamin B and showed similar changes as the deficient group (fig. 1) renders it highly improbable that a lack of vitamin B produces any specific deleterious effect on the blood forming tissues. The readiness with which regeneration of hemoglobin occurred in both control and deficients rats is apparent from figure 1, the 6th and 7th weeks of the experiment.

Blood sugar. Figure 2 presents graphically the data obtained on both control and deficient rats during vitamin B deficiency and recovery. The graph was made from a table of averages. It may therefore be of interest to insert some individual blood sugar values obtained on several rats from week to week throughout the experiment.

Rat 1: 119, 114, 103, 147, 134, 113, 132, and 146 mgm./100 cc.

Rat 4: 129, 137, 116, 150, 166, 133, 141, and 161 mgm./100 cc.

Rat 14: 120, 117, 112, 139, 154, 120, 160, and 134 mgm./100 cc.

It is clear that the values are erratic in all cases. Similar fluctuations in the blood sugar level were found in the water and food control rats in the present series. This fact appears to indicate that the changes observed are the result of inanition and not due to any specific effect of a lack of vitamin B. This seems all the more likely because of the fact that equally significant variations of the blood sugar level were evident when very rapid gains in weight occurred during the realimentation period of both control and deficient animals.

The question of corpuscular sugar is unsettled (Stammers, 1926). Some believe that glucose is equally distributed between plasma and corpuscles. If this view is accepted, then an increase in blood concentration should not materially decrease the reducing substance present in a given unit of whole blood. On the other hand, if the view is taken that the corpuscles do not contain an appreciable amount of reducing substance, then even a normal value for the whole blood sugar, in the presence of reduced plasma volume (anhydremia) would signify an absolute hyperglycemia. However, the theme of this paper is primarily concerned with the question: does vitamin B play any rôle in the regulation of the blood sugar level? The data permit a definite answer for the following reason. The blood sugar changes observed in the "water- and-food control" rats, which received their full quota of vitamin B daily, *and* developed anhydremia to approximately the same extent as the deficient animals, did not differ significantly from the results obtained in the deficient rats. Hence, vitamin B *per se*, can hardly be a factor directly influencing the blood sugar level under the conditions of these experiments.

Blood chlorides. The averages of all the data obtained are presented graphically in figure 3. There appears to be a definite hypochloremia in the rat during the greater part of the period it is kept on a ration deficient in vitamin B, although there is a tendency to a slight return to the

normal level during the advanced stage. In view of the fact that the changes in the blood chlorides of control rats do not differ radically from those seen in the the deficient animals it is likely that the effect observed is a result of inanition. Similar changes in chlorides were reported by Morgulis and Edwards (1924) in starvation. An examination of the blood chloride values obtained on individual rats reveals only moderate changes, but in general the tendency to a decrease is unmistakable. In no case during the vitamin B-deficient period was a blood chloride value obtained that was above the original normal level. On the other hand, a marked

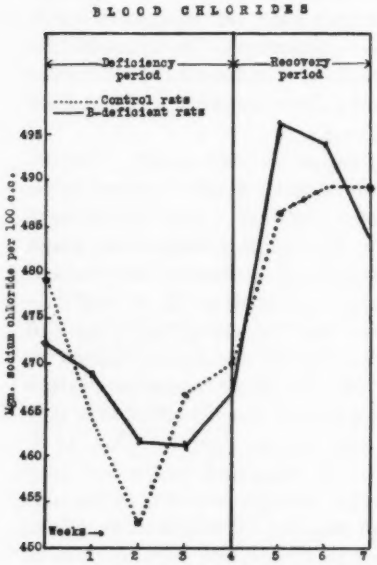

Fig. 3

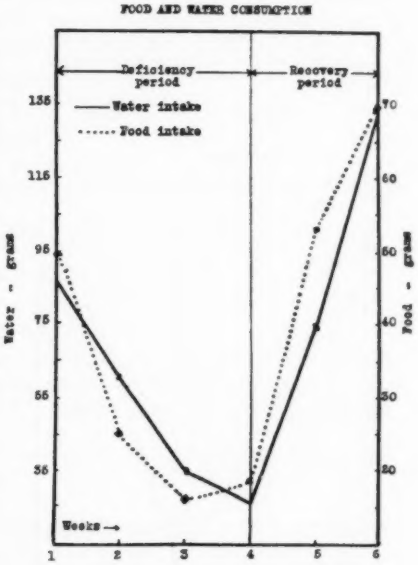

Fig. 4

increase in the chlorides of both deficient and normal rats was found during the first week of realimentation (fig. 3).

There are at least two reasons why one should expect the blood chlorides to be reduced in these animals. First, the work of Gamble, Ross and Tisdall (1923) suggests that a selective excretion of the chloride-ion occurs in starvation to supply base for the maintenance of the $BHCO_3$ at a nearly normal level. In the current experiments the animals manifested anorexia, so that starvation existed as a definite complication of B-avitaminosis. Secondly, evidence of the existence of anhydremia has already been given. It is well known that the chloride concentration of the corpuscles is less than that of the plasma. Hence, in the presence of reduced

plasma volume, although there may be a compensatory increase in corpuscles, the net effect would be a decrease in whole blood chlorides in dehydration.

Water and food consumption. Investigators are unanimous in the opinion that the food intake is diminished in B-avitaminosis. However, except for a recent communication (Stucky and Rose, 1929), no literature dealing with the fluid consumption in this deficiency disease has been found. Figure 4 shows the progressive and almost parallel decrease in water and food ingestion during the deficiency period. The points on the graph were obtained from the average food and water intake of all the vitamin B deficient rats, during each week of the experiment. The parallel increase in food and water intake during recovery is also evident from figure 4.

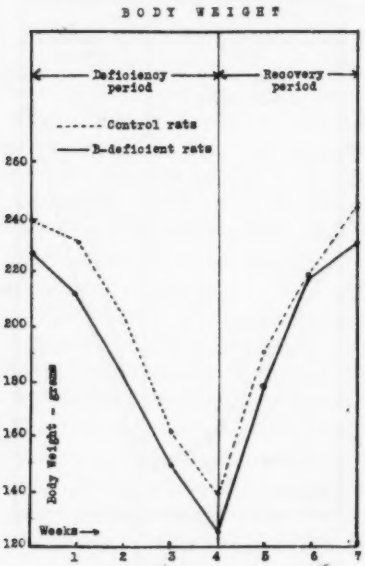

Fig. 5

Changes in body weight. The decline in body weight appears to be rather uniform and progressive (fig. 5). In this respect the graph resembles that showing the food and water consumption (fig. 4) and suggests that the change was largely, if not entirely, due to starvation incident to B-avitaminosis, rather than to any specific effect of a lack of this dietary factor. This viewpoint is supported by the fact that similar changes occurred in the control rats (fig. 5) which were given, on an average, the same amount of food and water, supplemented by a vitamin B preparation (400 mgm. of dried brewery yeast per rat per day).

Discussion. Evidence has been presented which seems to indicate that anhydremia occurs in vitamin B-deficient rats. The problem as to how a lack of vitamin B in the diet influences blood concentration now commands attention. In this connection, the results obtained in the experiments on rats are of special interest when compared with those obtained on dogs (Stucky and Rose, 1929). Thus when the extent of anhydremia in a control dog was practically the same as that of the corresponding deficient animal, prolonged loss of appetite in the latter was a conspicuous feature. Similarly, in the rat experiments the degree of blood concentration of control animals was essentially the same as that observed

in the vitamin B-deficient group *and* the starvation phenomenon—an outstanding characteristic of B-avitaminosis in this species—predominated. On the other hand, when a vitamin B-deficient dog ate fairly well, it developed anhydremia to a *greater* extent than its corresponding food and water control. However, in spite of this equi-caloric intake, the deficient animal manifested a more marked loss of body weight than the corresponding control dog.

In the case of the rats, the loss in body weight of both control and deficient animals was practically the same, and the degree of anhydremia manifested by both groups showed very little difference. These facts tend to support the suggestion made in our earlier experiments that the development of anhydremia in B-avitaminosis seems to be related to a loss in protoplasmic mass, rather than to any specific and direct effect of a lack of vitamin B.

TABLE 1

Vitamin B deficient ration

	PER CENT
Meat residue*	18
Corn starch	50
"Crisco"	23
Cod liver oil	5
Salt mixture**	4

* This substance is practically free from vitamin B (G. R. Cowgill. 1926. THIS JOURNAL, lxxix, 341). It may be obtained from the Valentine Meat Juice Company, Richmond, Va.

** The salt mixture described by Osborne and Mendel (Journ. Biol. Chem., 1917, xxxii, 374) was employed.

An examination of the blood sugar values on any given rat showed marked variations from week to week during the deficient and recovery periods. These results are in accord with those obtained by Eggleton and Gross (1924). The fact that changes of equal magnitude were obtained in control rats in the present investigation is in harmony with the observations of Mattill (1923) who concluded from his respiratory quotient studies on vitamin B deficient rats that this vitamin was not specifically related to carbohydrate metabolism. When averages of all the weekly values from blood sugar determinations are considered, it appears that a moderate hypoglycemia followed by a hyperglycemia occurs (fig. 2). This confirms the work of Drummond and Marrian (1926), except for the fact that similar changes were observed in the water and food control rats, which received adequate supplements of vitamin B daily. This emphasizes the importance of controlling the nutritive state when making blood sugar studies. It is well known that starvation diminishes the

tolerance to carbohydrates. The starvation phenomenon predominates to a greater extent in vitamin B-deficient rats than dogs. This may possibly be a partial explanation for the erratic blood sugar values obtained in the rats, which were not observed in the experiments on dogs (Stucky and Rose, 1929).

Anhydremia is a condition in which the water content of the blood is diminished. The movements of electrolytes and water cannot be divorced from each other. In the absence of data on the possible changes in other acid radicals of the blood in these rats, it is impossible to state to what extent the chlorides were replaced by other acid ions, and to what extent this decrease in chlorides was due to the anhydremia. Figures 1, 3 and 4 show very definitely how, during the first week of realimentation, the water intake and blood chlorides increased very definitely, while the hemoglobin (blood concentration) decreased considerably—suggesting an inverse relationship between the hemoglobin values and those for blood chlorides under conditions of dehydration. Similar changes were observed in vitamin B deficient dogs (Stucky and Rose, 1929). Clinically, chlorides are usually increased in anemia and decreased in anhydremia.

Perhaps the first evidence that anorexia occurs in vitamin B-deficient rats was presented by Osborne and Mendel (1917). In the current experiments the additional observation was made that the deficient animals also drink less. The mechanism through which a lack of vitamin B affects the appetite remains one of those subtle problems with which physiologists are often confronted. If animals are deprived of water, they refuse to eat. Recently, it was demonstrated that even short periods of water deprivation resulted in anorexia and complete gastric atony in dogs (Rose and Stucky, 1928). In the experiments herein reported the decrease of water and food consumption parallel each other so closely (fig. 4) that it becomes well-nigh impossible to venture a plausible suggestion as to which is primary.

If it be assumed that anorexia is primary then it is not difficult to understand why the fluid intake of the organism should be lessened. During starvation, the animal obtains most of its energy first from stored fat and later from the breakdown of protoplasm, the water content of which is considerable. To this metabolic water must be added.

An observation made during the course of these experiments perhaps favors the view that anorexia for the deficient diet is primary. It was striking to watch some of these ill-nourished, wretched looking deficient rats devour the yeast given them after they had refused to partake of the basal ration for weeks. This seems to indicate that they still maintained their appetite for material rich in vitamin B even in the advanced stages of the deficiency disease.

While the starvation picture predominates to a greater extent in vitamin

B-deficient rats than in dogs, this does not necessarily indicate that a species difference exists. It is now generally conceded that vitamin B is composed of at least two factors. One of these factors is believed to be "appetite stimulating." In the experiments on dogs vitamose[4] was used as a source of vitamin B, while the rats were fed dried brewer's yeast during the preliminary observation periods. The proportion of the components of vitamin B in these two vitamin concentrates are known to be different (Sherman and Axtmayer, 1927; Chick and Roscoe, 1927). In other respects the diets were not identical. Before it can be concluded that a species difference exists these experiments will have to be repeated with diets in which the exact content of each factor of the complex called vitamin B is known. Furthermore, it is not unlikely that the nutritive history of some dogs may be such as to provide them with a greater vitamin reserve with respect to the "appetite stimulating" factor. Until more is known concerning the respective physiologic properties of each of the components of vitamin B in relation to appetite, no dogmatic statement will be possible.

SUMMARY—CONCLUSIONS

Albino rats weighing between 198 and 264 grams were fed a basal vitamin B-deficient ration. Their water as well as their food intake was measured. A control group of animals was given on an average the same amount of food and water as the deficient rats consumed. In addition the controls were given adequate daily supplements of material rich in vitamin B.

Blood samples were obtained by cardiac puncture from each rat of both groups from week to week during the course of the vitamin deficient and realimentation periods. The blood was analyzed for hemoglobin, sugar and chlorides, employing micro-technique throughout.

Evidence of the development of anhydremia, as indicated by a definite increase in hemoglobin during the deficient period, followed by a pronounced reduction in this constituent during the first week of realimentation, was obtained. Changes observed in the control rats were commensurate with those found in the deficient animals. Hence it would appear that the phenomenon just described may be accounted for by the diminished food and water consumption incident to B-avitaminosis. The suggestion made in a previous communication (Stucky and Rose, 1929) that the tendency to anhydremia in vitamin B deficient dogs seems to be related to loss of protoplasmic mass, finds support from these experiments.

The data obtained point to the fact that no significant difference in the blood sugar level of control and deficient rats occurs. This appears to eliminate any specific influence of vitamin B in regulating the blood sugar level. Values for individual rats are erratic. Averages of all the blood sugar data during each week suggest a slight hypoglycemia followed by a

[4] Obtained through the courtesy of E. R. Squibb & Sons, New York, N. Y.

moderate hyperglycemia during vitamin B deficiency. However, similar results were obtained in the case of the controls. It seems, therefore, that the changes are due largely, if not entirely, to inanition.

There is a tendency to a decrease in blood chlorides in B-avitaminosis. Similar changes were observed in the food and water control animals. These results are in accord with those found in vitamin B deficient dogs—and are probably a result of starvation.

Evidence is presented indicating that there is a diminished water consumption in rats fed a diet deficient with respect to vitamin B. It is impossible to state at present whether this can be completely accounted for by the accompanying anorexia.

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STUDIES IN THE PHYSIOLOGY OF VITAMINS

X. Further Contributions to the Study of Gastric Motility in Vitamin B Deficiency[1]

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Although Morat (1882) was the first to record gastric contractions in unanesthetized dogs, it was Cannon and Washburn (1912) who presented the earliest convincing demonstration that the motor function of the stomach was related to appetite. The latter showed that the sensation commonly designated as "hunger pangs" was synchronous with vigorous gastric motility. It is now an established fact that anorexia is an early and prominent symptom of vitamin B deficiency in certain species (non-ruminants), and Ohomori et al. (1922) reported anorexia and gastric stasis in human beriberi. These facts make it highly desirable to determine whether or not the loss of appetite incident to B-avitaminosis has its physiologic basis in a disturbance of gastric motility in this deficiency disease.

In 1926 Cowgill and associates reported that "in mild cases of vitamin deficiency associated with anorexia there is no remarkable change in the character of the hunger contractions. The rhythmic tonus is frequently absent and the number of contractions in a series is somewhat less. In the severe cases of this deficiency in which anorexia is associated with nervous and muscular symptoms there is gastric atony." On the other hand, Smith (1927) reported that there seems to be some delay in the emptying time of the stomach in B-avitaminosis and that "there is an active peristalsis of the empty stomach, records of which cannot be distinguished from those of control animals." In a recent study of the effect of insulin on gastric motility in vitamin B deficiency, Stucky, Rose and Cowgill (1928) found a *decrease* in gastric motility in 6 out of 8 cases; in the two dogs which failed to show this decrease, considerable leakage from the gastric fistulae occurred, and abnormally vigorous contractions were recorded; one of

[1] These experiments are included in a preliminary report given before the Physiological Society of Philadelphia, December 17, 1928.

The data in this paper are taken from a dissertation submitted by William B. Rose in partial fulfilment of the requirement for the degree of Doctor of Philosophy, Yale University, 1928.

[2] Porter Fellow of the American Physiological Society, 1926–27.

these animals developed severe anemia and the other suffered from a dermatologic condition which was never diagnosed.

In view of this conflicting evidence, a study of the hunger contractions in B-avitaminotic dogs was undertaken, employing the following modifications in procedure:

1. Non-fistula dogs were used it being obvious that a gastric fistula is not strictly physiologic.

2. Parallel blood studies were made from time to time to control possible complications, e.g., anemia, hyperglycemia.

3. A group of control dogs received the same amount of food and water daily that the corresponding vitamin B-deficient animals consumed; in this manner an attempt was made to duplicate, as far as possible, nutritive changes manifested by the deficient dogs due to their variable but voluntary intake of food and water. A lack of vitamin B[3] in the diet thus would be the only known dietary variable operating.

4. A technique was perfected allowing one to record conveniently hunger contractions over long periods of time (continuously for 24 to 36 hours). It was reasoned that if no hunger contractions were present over a continuous period of 24 hours or longer the gastric atony could not possibly be attributed to a mere prolongation of the quiescent period.

5. Special care was observed to avoid taking gastric records on animals exhibiting *any* outward manifestations of vitamin B deficiency, except anorexia and a variable decrease in general activity; hence the gastric atony which developed in certain cases cannot be ascribed to a moribund condition of the animal at the time the gastric records were obtained.

General technique, feeding and diets. Dogs were used for all the experiments reported in this series of investigations. The animals were placed under observation for at least two weeks before the experiments were begun. During this period attention was paid to their general activity, their willingness to eat the "synthetic" ration and their behavior in other respects. Only active animals in good nutritive condition and which appeared particularly well adapted for these experiments were chosen. The animals were kept in individual cages and were bathed frequently.

The casein diet III described by Cowgill (1923a) was employed as the basal vitamin B deficient ration. Cod-liver oil was administered at frequent intervals to insure an adequate supply of vitamins A and D. Water was furnished ad libitum. Each animal was given 15.3 grams of the basal ration per kilo of body weight calculated on the basis of the initial weight, this quantity being sufficient to maintain constant weight (Cowgill, 1923b). The food was offered daily in one portion throughout the course of the experiment, regardless of whether the animal had eaten all of the previous day's allotment or not.

[3] This term refers to the vitamin B complex (Smith, 1928).

During the preliminary observation period each animal was given 0.6 gram of vitavose[4] per kilo of body weight per day (Cowgill, Deuel and Smith, 1925). The vitamin preparation was fed separately from the rest of the ration in order to avoid any possible influence on the palatability of the artificial food mixture.

In addition to vitavose, other preparations of vitamin B such as yeast vitamin powder (Harris)[5], vegex[6], and tomato juice were employed, mainly as therapeutic agents in the advanced stages of this vitamin deficiency.

The animals were fed once daily. On days when gastric motility records were taken or when blood was drawn for analysis, the feeding was delayed until after these procedures were completed.

The animals were divided into two groups: *a*, those fed the vitamin B-deficient ration described above; *b*, controls fed the same diet plus vitavose (0.6 gram per kilo per day). The latter were started on the experiments two days later than the animals in the deficient group. Water was given ad libitum only to the vitamin B-deficient dogs. Food and water pans were suspended securely in the cages in order to prevent any loss by spilling. By means of a control water pan placed in the animal room, the amount of water evaporated per day was noted. Due allowance was made for this factor when the water consumed by the deficient animals was recorded. *Each control dog was given the same amount of food and water, per kilo of body weight, that the corresponding vitamin B deficient animal consumed.* In addition, each control received its full quota of vitamin B regardless of its daily food allotment. The animals in this group were carefully paired as regards weight, age, stature and general activity, with animals in the vitamin B deficient group.

METHOD OF RECORDING GASTRIC MOTILITY. The apparatus in which the dog was placed was essentially a modified metabolism cage having a partition in the anterior portion. This partition consisted of movable upper and lower sections, each provided with a semi-circular notch padded with felt. When the dog was placed in this apparatus, the position of the lower section was regulated to suit the height of the animal. Then the upper section was lowered and fastened in position with hooks. Thus the dog's head extended through a circular padded orifice in the partition. The diameter of this opening was adequate to permit freedom of movement to such an extent that guy ropes had to be attached to the animal's collar to prevent it from withdrawing its head. With this arrangement the dog could conveniently stand in its habitual posture as in a cage. If fatigued,

[4] Obtained through the courtesy of the Ward Baking Company, New York City and E. R. Squibb & Sons, New York City.

[5] From the Harris Laboratories, Inc., Tuckahoe, New York.

[6] This is a concentrated vegetable extract made from grain grown yeast. It may be obtained from the Vitamin Food Co., Inc., Westfield, Massachusetts.

it could readily sit down without disturbing the tracing. Any desire to defecate or urinate was spontaneously effected without any apparent inhibition.

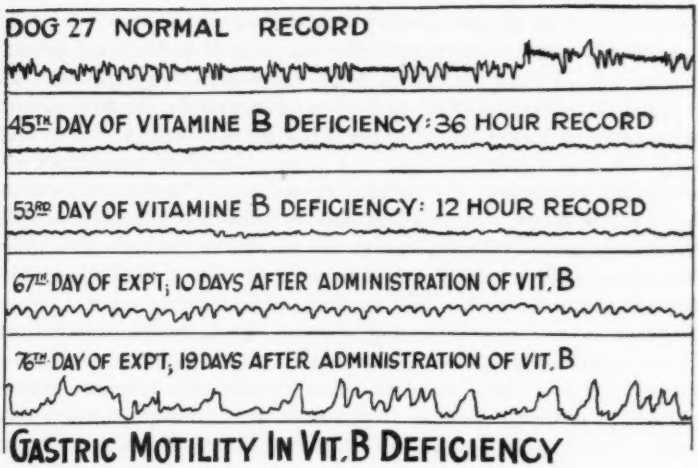
The animals were required to swallow a stomach tube having a thin rubber balloon tied to its distal end. In order to prevent the dogs from biting the tubing, a special bit was devised which had a central perforation through which the stomach tube was inserted. This tube was allowed to escape from the mouth through the natural spaces between the teeth of the upper and lower jaws. The balloon was then inflated, and the proximal end of the stomach tube attached to one arm of a chloroform manometer. The rest of the technique was essentially the same as that described by Cowgill and collaborators (1926). The other arm of the manometer carried a cork float provided with a fine glass stem, the upper end of which supported a glass writing pen. Changes in intragastric pressure were transmitted to the manometer, and recorded on an "endless" roll of white paper moved by a Cambridge kymograph driven by an electric motor.

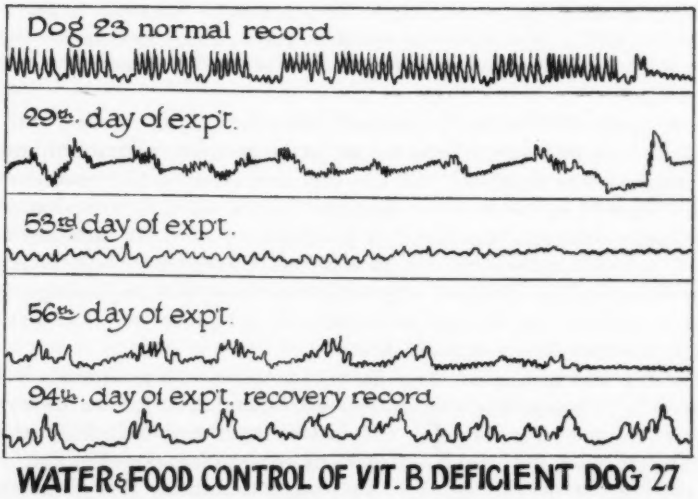
When very long records were taken, and the dog manifested a desire to lie down after standing for a few hours, it was placed in a hammock arrangement connected with the apparatus. The complete lack of discomfort on the part of the animals was evidenced by the fact that very little vigilance was required on the part of the operator even when a continuous tracing lasting 24 hours or more was taken. Indeed, the dogs not infrequently went to sleep during an experiment.

EXPERIMENTS. The results are characterized by a certain degree of variability. The effects on gastric motility observed in one animal differed considerably from those obtained in another. More remarkable is the fact that even greater differences were found in the control dogs, fed the same diet plus vitamin B. The diversity of the results serves to emphasize the importance of using a sufficiently large number of animals. These multiform effects on gastric motility may possibly be due, in part at least, to factors beyond present experimental control. Although the dogs were kept under observation for several weeks (during which period they were fed a complete diet) before the experiments were begun, beyond this their nutritive and biologic histories were not known. One significant factor contributing to the variability of the results obtained has already been investigated and will be discussed in a subsequent paper (Rose, Stucky and Cowgill, 1929). It apparently involves the regulation of blood concentration.

From what has already been said, it is clear that a "case" representative in every respect cannot be presented. The reader will therefore be prepared for a brief account of the studies on individual animals.

Vitamin B-deficient dog 27. Representative portions of some of the gastric records of this animal are illustrated in chart 1.


DOG 27 NORMAL RECORD
45TH DAY OF VITAMINE B DEFICIENCY: 36 HOUR RECORD
53RD DAY OF VITAMINE B DEFICIENCY: 12 HOUR RECORD
67TH DAY OF EXPT; 10 DAYS AFTER ADMINISTRATION OF VIT. B
76TH DAY OF EXPT; 19 DAYS AFTER ADMINISTRATION OF VIT. B
GASTRIC MOTILITY IN VIT. B DEFICIENCY


Dog 23 normal record
29th. day of expt.
58th day of expt.
56th day of expt.
94th. day of expt. recovery record
WATER & FOOD CONTROL OF VIT. B DEFICIENT DOG 27

Charts 1 (upper) and 2 showing representative gastric records of vitamin B-deficient dog 27 and its water-and-food control dog 23. The gastric tracings here reproduced are approximately one-seventh the original size.

The normal records of the hunger contractions of this dog revealed physiologic changes in gastric tonus, good amplitude and well sustained contractions.

On the 17th day of the experiment the dog was active and in excellent physical condition; gastric motility was normal.

For six days (39th to 44th inclusive) the animal had refused to eat the deficient diet. Except for this anorexia, there were no symptoms. However the gastric tracing (45th day) taken continuously for 36 hours failed to show any evidence of gastric tonus or hunger contractions. The tracing proved to be essentially a straight line indented only by respiratory movements which were practically uniform throughout. This indicates that the technique was sensitive to small changes in intra-gastric pressure. Moreover, the regularity of the respiratory movements and the cheerful attitude of the animal afford striking confirmation of the fact that the method was satisfactory even for such a long continuous gastric tracing.

At this stage of the experiment gentle tugging on the stomach tube with the inflated balloon attached to its distal end failed to initiate hunger contractions. This stimulus almost invariably elicited a response in normal dogs during gastric quiescence. Apparently the brunt of such stimulation is borne by the cardiac end of the stomach. In this connection the work of Alvarez (1916; 1917) deserves mention. He pointed out that the cardia was the most irritable portion of the stomach. Likewise, Rogers and Martin (1927), in their x-ray studies state that "peristaltic waves have been seen to originate high up in the fundus, at or near the cardiac sphincter."

It is significant that neuromuscular symptoms did not develop until *ten days after* the first long tracing revealing the complete absence of hunger contractions was obtained. On the 49th day of the experiment another record showed gastric atony. However, on the 53rd day of vitamin B deficiency, occasional variations in gastric tonus of short duration were noted, but no definite contractions occurred. Three days later a similar gastric tracing was obtained. By this time the animal had become very inactive and continued so until neuromuscular symptoms developed at the end of the 56th day of experiment.

The dog was then given large doses of material rich in vitamin B and recovered. The appetite was rapidly regained and almost twice the normal food requirement was allowed. It is interesting to note that this food was quickly eaten yet the hunger contractions were shallow as seen in chart 1 on the 67th day of the experiment. This seems to indicate that under these circumstances the apparent avidity for food is not directly related to the vigor of the hunger contractions of the stomach. Nevertheless it should be added that while the contractions were small, the period of total gastric activity was, if anything, somewhat greater than normal. On the 19th day after the administration of vitamin B very vigorous contractions were present, but even then the motor activity of the stomach did not appear to be quite normal. However three weeks later a continuous 24 hour

tracing was obtained which revealed excellent contractions, normal sequence, well-sustained tonus and good variation in gastric rhythm. Thus it seems that the return to the physiologic state following vitamin B therapy is a gradual process.

Control dog 23. This animal served as a control on the food and water consumption of vitamin B-deficient dog 27, the gastric tracings of which have just been described.

A brief résumé of the significant changes in gastric motility observed in control dog 23 is exhibited in chart 2.

The early tracings taken on this animal were essentially normal. On the 53rd day of experiment there was evidence of a definite decrease in gastric motility, in spite of the fact that this animal continued to receive its full quota of vitamin B daily. However, fairly vigorous contractions were present three days later. An examination of the protocol showed that this animal received considerably less water when the former record was taken. This, of course, was a result of the fact that the corresponding experimental dog 27, lacking vitamin B, voluntarily drank less water during the same period. No such changes were observed in normal dogs allowed fluid ad libitum whose gastric records were studied for months. A partial explanation for this phenomenon will be clear from experiments dealing with gastric motility in dogs deprived of water (Rose and Stucky, 1928).

At this stage of the experiment (56th day) the corresponding vitamin B-deficient dog 27 revealed atony of the stomach recorded for over 50 hours. Furthermore, the deficient animal developed neuromuscular symptoms and was quite ill. In sharp contrast, the control animal (dog 23) was in good physical condition in spite of 18 days of starvation (and partial water deprivation) and manifested fairly good gastric motility. This animal was realimentated and all the recovery records revealed definitely physiologic tracings.

In comparing the changes recorded in these two animals, it is difficult to escape the conclusion that a lack of vitamin B in the diet accentuates the disturbances in the motor function of the stomach, resulting from a decreased food and water intake.

Vitamin B-deficient dog 25. The tracings taken early in the experiment showed vigorous contractions, good gastric tonus and normal rhythm.

This animal lost its appetite for the deficient ration on the 29th day of the experiment. There was a definite decrease in fluid intake and an apparent decrease in general activity. However, except for anorexia, and the presence of vomitus in the cage on the 42nd day, no external symptoms were evident. The gastric tracing at this time revealed definite evidence of abnormality. There was a pronounced disturbance in sequence as manifested by marked prolongation of the quiescent period. The hunger

contractions were fewer and of smaller amplitude; gastric tonus was poor.

A tracing taken two days later (44th day of experiment) showed quite vigorous contractions for the first hour. Subsequently, the contractions were small, and irregular in occurrence. The dog was very quiet but symptoms, other than anorexia, were conspicuous by their absence.

On the 47th day of experiment extremely shallow contractions were obtained at irregular intervals. Small tonus changes still persisted. On the following day slight muscular incoördination made its appearance—hence no gastric tracing was taken.

This experiment is of special interest. It will be recalled that vigorous contractions—practically normal—were present for about one hour on the 44th day of B-avitaminosis, although the rest of the tracing showed marked depression of gastric function. The animal died only six days later following severe convulsions. Had the records of this dog been restricted to very short tracings, the limited data thus obtained would agree with those reported by Smith (1927) who stated that the hunger contractions observed in dogs showing initial symptoms of beriberi were not distinguishable from those of normal dogs. It is obvious therefore that unless tracings of considerable length and of sufficient frequency are taken on the same animal, it is unsafe to draw conclusions. In support of this viewpoint, the results of one tracing on dog 31 may be mentioned here. On the 32nd day of vitamin B deficiency, this dog's tracing showed four hours of gastric activity, in a 24 hours' period. In the remaining twenty hours hunger contractions were practically absent.

Control dog 21. This animal was given its daily requirement of vitamin B, but its food and water intake was limited to that of deficient dog 25. The latter did not eat any of the deficient food for 21 days and died. The control remained very active throughout, but the gastric records showed a progressive decrease in motor activity of the stomach which was remarkably similar to that of the deficient animal during the control period.

Thus on the 27th day of experiment the normal sequence appeared somewhat disturbed; most of the contractions were smaller than normally, except towards the end of the 18th hour, when one set of vigorous contractions, associated with well sustained tonus, was noted. It was quite apparent that the total period of activity of the stomach's musculature was lessened.

By the 50th day of experiment this animal had been deprived of food for 21 days, and also received less water than it would voluntarily consume. This was evident from the fact that the quantity of water supplied daily was often consumed within one hour or less.

The changes in gastric motility at this time were 1, definite decrease in activity but gastric atony was absent (note: the deficient animal did not

develop gastric atony either); 2, a comparison with the earlier records made it obvious that the changes were more pronounced; no vigorous hunger contractions were present at any time; small periods of activity were associated with shallow contractions; the quiescent time was definitely prolonged.

When the B-deficient animal died the control was given adequate food daily and water ad libitum. Gastric records taken at intervals during realimentation over a period of a few months showed that the normal

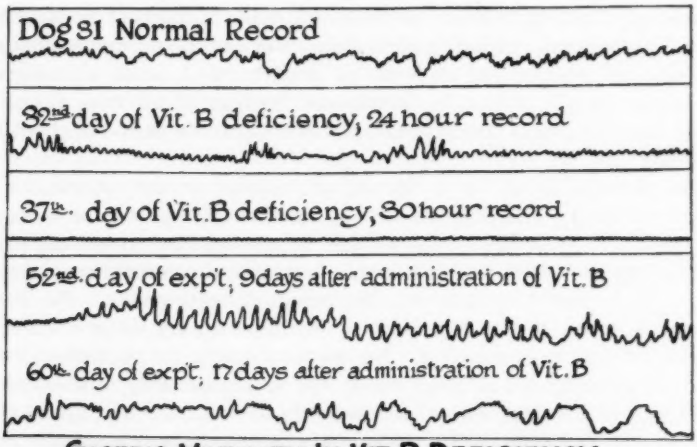

Dog 31 Normal Record
32nd day of Vit. B deficiency, 24 hour record
37th day of Vit. B deficiency, 30 hour record
52nd day of expt, 9 days after administration of Vit. B
60th day of expt, 17 days after administration of Vit. B
GASTRIC MOTILITY IN VIT. B DEFICIENCY

Chart 3. Extracts from gastric tracings of dog 31, while subsisting on a diet deficient in vitamin B, and after receiving the missing dietary factor. On the 37th day of the dietary deficiency regime no evidence of gastric contractions was obtained over an observation period of 30 hours; atony had developed. The effect of administration of vitamin B on the depressed motility is obvious. The tracings here reproduced are approximately one-seventh the original size.

physiologic activity of the stomach returned as the animal regained its original nutritive state.

Thus a dog that received its adequate requirement of vitamin B daily showed disturbances in gastric motility comparable to those found in the corresponding deficient dog 25 and apparently due to a diminished food and water consumption. Carlson (1916) claims that starvation alone accentuates gastric activity, but in this case the water intake was limited to that consumed voluntarily by the B-deficient dog, as already mentioned. The possible significance of this factor has been investigated; as already stated, the data of the latter study will be found in a subsequent paper (Rose, Stucky and Cowgill, 1929).

Vitamin B-deficient dog 31. A few typical portions of various gastric records of this animal are depicted in chart 3.

Tracings of the motility of the stomach of this dog are particularly instructive because they demonstrate in a beautiful manner the gradual transition from normal gastric activity to partial loss (e.g., one continuous 24 hour tracing on 32nd day of experiment in which contractions were only present for 4 hours) and then finally to complete gastric atony *recorded* for over 100 hours and extending over a period of ten days. Conversely, upon realimentation, there is a change of state of the gastric musculature as evidenced by improvement in tonus and vigor of the contraction (52nd day of experiment, chart 3) and finally a return of the normal sequence of contractions and quiescence with all gradations of tonus changes and occasional gastric tetany, in subsequent tracings which were taken for many hours at a time (10–24 hours) at intervals over a period of about two months.

The physical condition of the animal throughout this experiment deserves mention. This dog was very young; its exact age was not known. Carlson (1916) has shown that hunger contractions are more vigorous in young animals. This lends more significance to the occurrence of gastric atony, particularly because the dog remained quite active. No neuromuscular symptoms were present at any time during the periods when gastric tracings were recorded. Except for anorexia for the deficient diet, and the development of small uninfected and apparently nutritional denudations of the skin, which have recently been commented upon (Cowgill, Stucky and Rose, 1929), the animal appeared to be in a normal physical state when pronounced disturbances in the motor function of the stomach were recorded. The blood sugar level remained well within the normal limits throughout the experimental period. This is worthy of note in view of the findings of Bulatao and Carlson (1924).

Vitamin B-deficient dog 28. The gastric motility of this dog showed excellent sequence and well sustained contractions with occasional evidence of gastric tetany. The animal itself was very vigorous and active and remained so until about the 50th day of B-avitaminosis.

The decrease in activity of the musculature of the stomach was somewhat gradual but the appetite remained fairly good. It is significant that hunger contractions were present the day prior to the occurrence of the first signs of nervous symptoms. Nevertheless the quiescent period was definitely prolonged and the contractions were small. This was evident on the 47th day of vitamin B-deficiency, and more pronounced by the 62nd day of the experiment. At this time the animal was very inactive. It developed an ulcer, the size of a 50 cent coin on the right buttock, the report of which was included in a recent article (Cowgill, Stucky and Rose, 1929).

On the 63rd day of experiment slight neuromuscular symptoms de-

veloped, hence no further observations on gastric motility were made. These symptoms became worse and convulsions developed which resulted in death.

In contrast to deficient dogs 27 and 31, which showed gastric atony approximately ten days or more before nervous symptoms developed, this dog had contractions on the day before muscular incoördination was first noted. A report of the changes observed in the water and food control of dog 28 now commands attention.

Control dog 24. As pointed out above, deficient dog 28 maintained a fairly good appetite, so that the control dog received almost adequate requirements of food and water daily. The latter did not lose any body weight. In spite of the artificial diet and confinement in the laboratory for a few months, the control maintained its normal activity, and on the 59th day of the experiment excellent hunger contractions with physiologic variations in rhythm and gastric tonus were recorded. In fact, on the 66th day of the experiment, when the corresponding deficient dog was moribund, the control manifested vigorous hunger contractions, indistinguishable from normal records except for the fact that the period of total gastric activity was perhaps slightly less. The gastric records of this animal speak favorably for the adequacy of the diet and trustworthiness of the method for recording gastric motility as well as the general technique. The slight prolongation of the quiescent period on the 66th day of experiment may possibly be explained by the fact that the water intake during the few days previously was considerably less, because the deficient dog 28 had neuromuscular symptoms during that period and drank less. On the first day of realimentation when water was given ad libitum, the control dog drank about twice the amount of fluid that it was wont to consume in 24 hours. As already mentioned, the effect of diminished water intake on gastric motility will be described in a succeeding paper of this series.

Vitamin B-deficient dog 26 (chart 4). By the 29th day of the experiment, there was a slight suggestion of some decrease in total gastric activity, although very vigorous contractions were obtained. One week later this was more evident. A continuous 24 hour tracing showed that in general the contractions were smaller and the quiescent periods were somewhat prolonged. The appetite was erratic at this time.

The physical condition of the animal remained good, except for a peculiar phenomenon—the ears were slightly tender to touch and obviously painful when pressed between the fingers. This tenderness persisted for weeks—without getting worse or revealing any anatomic changes, or apparent cause for this condition. After vitamin therapy the phenomenon gradually disappeared.

On the 47th day of the experiment there was definite evidence of further decrease in the frequency and vigor of the hunger contractions. This was

much more pronounced by the 59th day of vitamin B deficiency. It should be added, however, that whereas all other gastric tracings were taken approximately 24 hours after food was offered this record was taken only 8 hours after the last feeding. The dog vomited. The vomitus gave clear evidence that the digestive function was impaired because the food was hardly altered in appearance. In spite of this gastric stasis, the animal continued to eat part or all of its daily food allotment during this phase of the experiment, and vomitus was often seen in the cage.

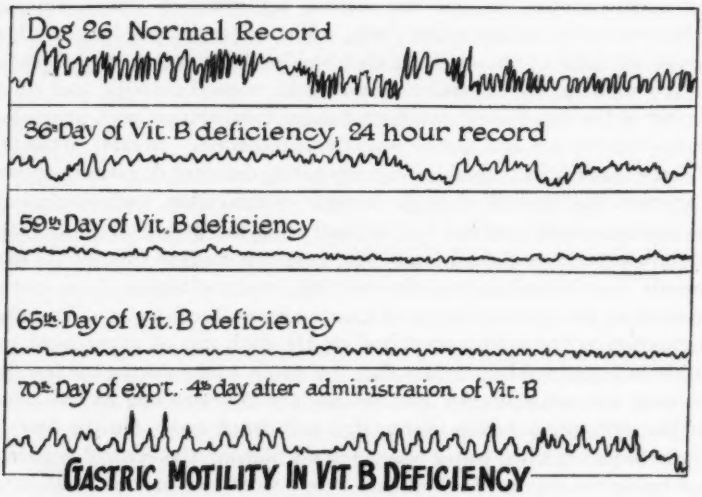

Dog 26 Normal Record
36th Day of Vit. B deficiency, 24 hour record
59th Day of Vit. B deficiency
65th Day of Vit. B deficiency
70th Day of expt. 4th day after administration of Vit. B
GASTRIC MOTILITY IN VIT. B DEFICIENCY

Chart 4. Representative gastric tracings obtained with dog 26. This animal subsisted on a diet adequate except for vitamin B for 66 days. Gastric atony appeared on the 59th day and lasted for a week. Marked improvement in gastric motility observed four days after administration of the missing vitamin is evident from the lowest tracing. The records here reproduced are approximately one-seventh the original size.

On the 63rd day of experiment, only a few aborted attempts at hunger contractions were registered; gastric tonus changes were practically absent. By this time the dog became much less active. Two days later the animal was very quiet and a gastric tracing revealed practically complete gastric atony. However no neuromuscular symptoms were seen (65th day of experiment). On the following day incoördination of gait appeared, which was soon followed by other nervous symptoms and vitamin B therapy was instituted.

Recovery was speedy—and more remarkable was the rapid return of

vigorous hunger contractions (70th and 71st days of experiment). The reader will recall no doubt that in the case of deficient dogs 27 and 31 gastric atony prevailed for approximately 10 days before nervous symptoms developed and the recovery of normal hunger contractions was rather slow. In the case of deficient dog 26, however, hunger contractions persisted until a few days before nervous manifestations were observed and recovery of the gastric vigor was more rapid.

Control dog 22. This animal served as the food and water control of dog 26. On the 64th day of the experiment, there was a decrease in the vigor of the contractions, and a definite prolongation of the quiescent period. The following day only one set of good contractions was obtained; the rest of the tracing revealed small contractions at irregular intervals. The cycle of gastric activity was apparently impaired. On this day the corresponding deficient animal manifested gastric atony.

Realimentation was followed by a rapid return to normal gastric motility.

Discussion. It is apparent that the effect of a lack of vitamin B on gastric motility in dogs is not quantitatively identical in any two animals. However, this variability in the data gives them additional value because it affords a partial explanation for the conflicting reports of previous investigators.

The portions of the tracings illustrated in the charts depict all gradations of disturbances in the muscular efficiency of the stomach. On the whole definite evidence of a decrease in gastric activity was obtained in *every* vitamin B deficient animal studied. The occurrence of complete gastric atony recorded for long periods of time (e.g., over 100 hours in dog 31) appears to present conclusive evidence that vitamin B in some manner (probably indirectly) plays an important rôle in influencing the motor function of the stomach. The finding of gastric atony in *some* dogs confirms the work of Cowgill et al. (1926). The criticism lodged against the work of the latter investigators, namely, that the animals were probably moribund when atony of the stomach developed, cannot hold, because in the present study no tracings were made after neuromuscular symptoms developed.

On the other hand, the fact that *some* dogs failed to show gastric atony a day or two before nervous symptoms made their appearance—in some instances these same animals yielded short periods of vigorous gastric activity approximately a week before convulsions set in,—partially supports the observations of Smith that hunger contractions of vitamin B-deficient dogs cannot be distinguished from those of normal animals. Nevertheless, even in these animals, when long records of the motor function of this organ are taken (continuous for 24 hours or more), it becomes apparent that the period of total gastric activity is definitely diminished in this deficiency disease. It should be added, however, that Smith (1927)

made it clear that the records she obtained were taken on dogs that refused the deficient diet for only four days, whereas in the current experiments gastric motility was studied in animals in which the depletion of vitamin B reserve was probably more pronounced. In fact, in some instances anorexia supervened for approximately three weeks.

In harmony with the finding of gastric atony in some dogs, is the report of dilatation of the stomach in rats maintained on a diet deficient with respect to vitamin B (Rowlands and Browning, 1928).

Although there seems to be some general relationship between appetite and gastric motility, the data make it impossible to decide whether a quantitative relationship exists. The animals frequently ate food which apparently remained in the stomach for many hours and was later vomited. The vomitus was usually undigested food, which was barely altered in appearance. This fact is of interest in the light of the work of Farnum (1926) who found that the secretion of hydrochloric acid was greatly diminished in experimental beriberi in the dog. It is likewise supported by the report of gastric stasis in clinical beriberi[7] (Ohomori et al., 1922). The observation was frequently made that during realimentation the appetite invariably returned long before the hunger contractions were normal in frequency and vigor. The appetite varied considerably in some dogs. They would eat during a given week and refuse food the next. However, although gastric records were not taken daily, the general impression gained from the available data was that the decrease in gastric motility was more uniform and progressive in character, whereas the appetite was often erratic.

Gastric atony was usually associated with complete anorexia for the deficient diet but complete loss of appetite was *not* invariably associated with gastric atony. One dog refused all of the deficient diet for 21 days and died, but did not develop gastric atony, at least before nervous symptoms developed.

Bulatao and Carlson (1924) and others have shown that there is some relationship between gastric motility and the blood sugar level, briefly, that hyperglycemia is associated with a decrease in the motor function of this organ whereas hypoglycemia increases the vigor of hunger contractions. A number of workers have reported hyperglycemia in vitamin B deficiency. However, the decrease in gastric motility observed in the present study cannot be attributed to hyperglycemia because in all cases throughout these experiments the blood sugar level remained well within the normal range.

The work of Thomas and Kuntz (1926) has demonstrated that the muscles of the stomach continue to contract after the external and internal nervous mechanisms have been destroyed. Hence the disturbances in

[7] Recently, Matsumura et al. (Journ. Amer. Med. Assoc., xcii, 1325, April 20, 1929) have claimed that beriberi is caused by a specific bacillus.

gastric motility herein described are probably not attributable to histopathology of the myenteric nervous mechanism as suggested by McCarrison (1921). The present study indicates that even mild neuromuscular symptoms may be entirely absent and gastric atony still prevail in *some* dogs. Thus atony of the stomach was recorded on two dogs, about ten days before the earliest nervous manifestations developed. Certainly the data obtained in this study leave no room for doubt that the gastric tracings of dogs suffering from vitamin B deficiency *can* be readily distinguished from those of normal dogs by the fact that, long before nervous symptoms occur, there is a variable but definite decrease in gastric motility and occasionally gastric atony in all uncomplicated cases.

Perhaps the most interesting fact brought to light by these experiments is the finding that the water and food control dogs (with one exception—dog 24) likewise revealed disturbances in gastric motility, in spite of the fact that they were given an adequate supplement of vitamin B daily. Hence, the impairment of gastric function cannot be attributed to a *specific* effect of the lack of vitamin B per se. Nevertheless, the fact that none of the control dogs developed gastric atony, indicates that the lack of this vitamin appears to accentuate the changes described. Just how this dietary essential acts is not clear, but the effect is probably not limited to the musculature of the stomach.

SUMMARY AND CONCLUSIONS

The occurrence of anorexia in vitamin B deficiency led to a search for its physiologic basis by studying gastric motility. A series of control dogs were employed, each of which received the same amount of *food and water* daily that was consumed by a corresponding deficient animal. A technique was developed which made it possible to conveniently record hunger contractions continuously over a period of 24 hours or longer. Records of gastric motility were made at frequent intervals during the course of vitamin B deficiency and recovery therefrom. Tracings were never taken when neuromuscular symptoms were present. The data appear to warrant the following conclusions:

1. Disturbances of gastric motility developed in all dogs suffering from vitamin B deficiency.

2. This depression of the motor power of the stomach may become so pronounced as to yield evidence of complete gastric atony (recorded for over 100 hours in dog 31) in *some* cases. Atony of the stomach may occur long before nervous manifestations are apparent (approximately 10 days in these experiments).

3. Complete anorexia for the deficient food over a period of weeks is not necessarily associated with gastric atony. Shallow hunger contractions and slight tonus changes have been recorded one day prior to the onset of nervous manifestations.

4. The avidity for food does not appear to depend upon the *vigor* of the hunger contractions under the conditions of these experiments.

5. The "water-and-food" control animals likewise showed evidence of a variable decrease in total gastric activity. However, none of the controls developed gastric atony.

6. The data appear to indicate that the decrease in gastric contractions is not due to any specific and direct effect of a lack of vitamin B in the diet. However, a deficiency of this food essential seems to accentuate the disturbances described. The blood sugar level remained well within the normal range in all cases, so that hyperglycemia does not appear to enter into the mechanism involved.

7. Evidence is presented to suggest that the water intake of the organism may be one of the factors which affects the muscular contractions of the stomach.

8. Gastric stasis was present in some vitamin B-deficient animals.

9. In general, conflicting reports of previous investigators are accounted for. The results obtained are in harmony with other facts in the literature pertaining to this subject, so far as the writers are aware.

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STUDIES IN THE PHYSIOLOGY OF VITAMINS

XI. The Effect of Insulin on Gastric Atony in Vitamin B Deficiency[1]

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Bulatao and Carlson (1924) found that insulin, administered parenterally, produced an augmentation of gastric tonus with an increase in the vigor of the hunger contractions of the stomach. Furthermore, they demonstrated that hyperglycemia resulting from glucose injections decreased gastric motility in normal dogs. This work has been confirmed by Mulinos (1927). Likewise, Simici and collaborators (1927) reported that intravenous injections of insulin into human subjects produced a marked exaggeration of the normal motility of the empty stomach. These investigators concluded that there is some relationship between the blood sugar level and the vigor of the hunger contractions.

In an earlier paper (Stucky, Rose and Cowgill, 1928) we reported experiments designed to determine the effect of insulin on gastric motility in vitamin B deficiency. Dogs with simple *gastric fistulae* were employed. It was found that "of 16 insulin experiments performed on dogs showing a decrease in gastric motility, 25 per cent yielded what appeared to be a positive response to the hormone as indicated by a definite improvement in the motility of the stomach."

The present communication is a report of work planned to determine whether insulin would improve gastric motility in *non-fistula* dogs manifesting *prolonged atony* of the stomach due to B-avitaminosis. Two of our animals exhibited this condition over a period of at least ten days before nervous symptoms set in. The insulin experiments here reported were performed on these two dogs.

Technique. The general procedure has been described in the preceding paper (Rose, Stucky and Cowgill, 1929). Non-fistula dogs were fed a basal vitamin B[3] deficient ration and frequent observations of gastric

[1] The data in this paper are taken from a dissertation submitted by William B. Rose in partial fulfilment of the requirement for the degree of Doctor of Philosophy, Yale University, 1928.

[2] Porter Fellow of the American Physiological Society, 1926–27.

[3] This term refers to the vitamin B complex (Smith, 1928).

motility were made. When *atony* of the stomach developed, insulin was injected and the effects on the gastric tracing recorded. Blood samples for sugar determination were taken before and after the administration of the hormone. Estimations were made according to the method of Hagedorn and Jensen (1923), duplicate analyses being made in all cases.

The hemoglobin and total solids of the blood were estimated occasionally, using the technique previously described (Stucky and Rose, 1929).

THE EXPERIMENTS. *Experiment 1, dog 31.* A record of the gastric contractions was taken on the 37th day of the vitamin B-deficient period. After a continuous tracing for 24 hours revealed complete atony of the stomach, the record was inter-

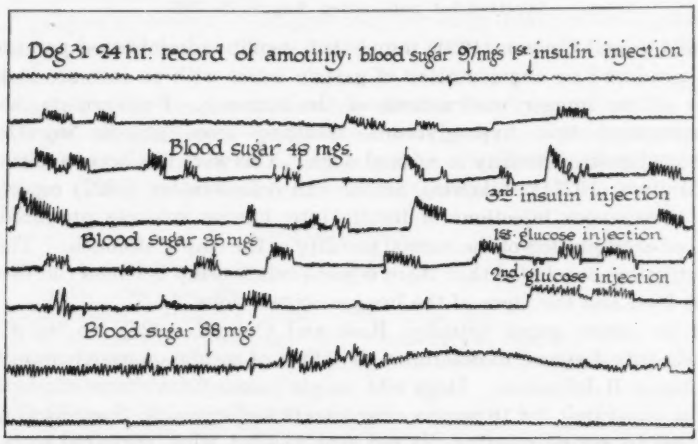
Dog 31 24 hr. record of amotility; blood sugar 97 mgs 1st insulin injection
Blood sugar 48 mgs.
3rd insulin injection
1st glucose injection
Blood sugar 95 mgs
2nd glucose injection
Blood sugar 88 mgs
EFFECT OF INSULIN ON GASTRIC ATONY IN VIT. B DEFICIENCY

Chart 1. Gastric tracings obtained with dog 31. The records here reproduced are approximately one-seventh the original size.

rupted for a while and the dog given water. It drank 60 cc. Two hours later a blood sample was taken. The blood sugar was found to be 97 mgm. per 100 cc. The gastric tracing was then continued. After the record had been obtained for approximately two hours without showing any evidence of hunger contractions, insulin was injected subcutaneously (two units per kilo of body weight) at 7:50 p.m. (38th day). Approximately one hour after the injection, occasional short, aborted attempts on the part of the stomach to contract were reflected on the tracing (chart 1). There was no variation in gastric tonus. The amplitude of the contractions was very small. These shallow and incompleted hunger contractions were very infrequent and disappeared completely within an hour.

A second insulin injection (2 units per kilo) was again made at 9:50 p.m. and its effect on the gastric motor mechanism observed. Some evidence of definite hunger contractions developed an hour later. These, however, were never as vigorous as normally; the physiological variations of gastric tonus, so characteristic of the

normal stomach, were practically absent at this time. The effect of the insulin was transient and disappeared within an hour. The blood sugar level was determined and was later found to be 48 mgm. per 100 cc.

Inasmuch as the response to insulin was not as pronounced as had been expected, and because it is well known that some dogs are much more susceptible to this hormone than others, a third injection of insulin (2 units per kilo) was made. Within a half-hour the animal appeared quite restless and blood was taken for sugar analysis. It was later found to be 35 mgm. per 100 cc. The response of the stomach to this injection was more pronounced than to either of the two previous injections. However, the dog became very restless and muscular twitchings were observed one hour later (12:50 a.m.). These manifestations were taken as a warning of impending hypoglycemic convulsions (as the low blood sugar value was not known until afterwards), and glucose was injected subcutaneously (65 cc. of 20 per cent glucose-saline). This injection was repeated again within an hour (2 a.m.), at the time when the animal appeared restless once more. Approximately three hours after the third injection of insulin (3:20 a.m.) there was a definite variation in gastric tonus. However, by this time, glucose had been injected twice and the blood sugar level had risen to 88 mgm. per 100 cc. Some definite contractions developed lasting about 15 minutes in spite of the fact that the blood sugar level had returned to normal. Small variations in gastric tonus persisted for about one-half hour and gradually died away. After an additional three hours of tracing, during which time gastric atony supervened, the experiment was discontinued. The animal showed no symptoms whatsoever, was active, lively and apparently in good physical condition.

Comment. The work of Bulatao and Carlson has been adequately confirmed by subsequent workers and is in accord with clinical experience. In cases in which an overdose of insulin produces hypoglycemia, the patients complain of severe hunger pangs. That the latter are related to hypermotility of the empty stomach has been shown by Carlson (1916). Hence, there can be no doubt that insulin injections in some way affect gastric motility although the exact nature of the mechanism of insulin action is far from clear. Consequently, the interpretation of the results of this experiment must necessarily be incomplete.

The presence of complete gastric atony in this case can not be seriously questioned. The effect of the first insulin injection was very slight. This seems to indicate that the gastric motor mechanism was apparently less responsive to insulin than normally, and affords some indirect evidence in support of the statement that gastric atony was definitely present and that this was not a case of "prolongation of the quiescent period."

The dosage employed was found to be effective and still within the zone of safety (Stucky, Rose and Cowgill, 1928). A number of investigators (Drabkin and Edwards, 1924; Best and Ridout, 1925; Drabkin and Shilkret, 1927) have reported the occurrence of anhydremia following the injection of large doses of insulin. In the experiments on gastric motility and anhydremia, following water deprivation (Rose and Stucky, 1928), it was found that anhydremia produces gastric atony.

Consequently, it was important to determine whether the three succes-

sive injections of insulin (using two units per kilo for each injection) produced anhydremia as well as hypoglycemia. Hemoglobin was estimated at intervals of one to two hours after each insulin injection. Occasional determinations of blood solids were also made. No significant change in blood concentration occurred even after the third injection. Hence, this possible factor cannot be invoked to account for the rather poor response of the stomach to insulin under the conditions of this experiment.

Part of this effect of insulin on gastric motility occurred when the value for the blood sugar was 35 mgm. per 100 cc. This is obviously at the convulsive level. Hence, the effect on gastric motility in this instance may possibly be part of a general stimulation of the muscular system as well as a local reaction on the part of the stomach itself.

From the results recorded above, it would appear that the gastric atony which occurs in vitamin B deficiency may be so pronounced in some dogs that even such a powerful physiologic agent as insulin fails to completely restore the hunger contractions, even after repeated administrations of the hormone.

The fact that the best variation in gastric tonus and also the fact that fairly definite hunger contractions were obtained *after* the blood sugar had returned to normal, indicates that the relation of insulin to gastric motility is not a simple one. The changes in the blood sugar represent only a part of the general systemic effect of insulin. That the hormone may affect other blood constituents (e.g., calcium, phosphorus, lactic acid, etc.) has been shown by various investigators (Briggs, Koechig, Doisy and Weber, 1923–24; Harrop and Benedict, 1924; Davies, Dickens and Dodds, 1926; Brougher, 1927; Katayama and Killian, 1927).

The evidence that some improvement of gastric motility developed after the blood sugar returned to normal cannot be attributed to the presence of vitamin B in the insulin, because it has been shown (Stucky, 1928) that this hormone does not contain any appreciable amount of this vitamin.

Gastric records were taken on dog 31 for several days after the animal had been given injections of insulin. There was no evidence of variation in gastric tonus during the course of these observations, many hours of gastric tracings having been taken. Not a single hunger contraction developed. Gastric atony was complete.

Experiment 2, dog 31. A second insulin experiment was performed on this dog on the 43rd day of vitamin B deficiency. The same dose (2 units per kilo) of insulin was injected subcutaneously.

Except for a slight rise in gastric tonus at intervals during the first hour after the injection, practically no change in gastric motility was observed. The animal became very restless three hours after the hormone had been injected. This response of the animal, usually considered to be due to hypoglycemic neuromuscular stimulation, was not associated with any significant effect on gastric atony. About

fifteen minutes later the dog suddenly became cyanotic and developed muscular twitchings similar to those present in the clinical picture of insulin overdosage (Harrop, 1927). Consequently, glucose (50 cc. of a 50 per cent solution) was injected. Vitamin B therapy was instituted and the animal recovered.

Comment. In this case, the degree of gastric atony was so marked that even insulin failed to produce any significant effect on gastric motility. It is repeated that the dog was active and except for anorexia, no external symptoms were present before the insulin experiment was begun. However, the injection of insulin seems to have precipitated the development of symptoms; this necessitated the administration of vitamin B-containing foods.

Experiment 3, dog 27. It is not infrequently observed in pharmacologic studies that an animal may exhibit an idiosyncrasy for a particular drug. Consequently, the effect of the injection of insulin on gastric atony was determined in another animal—dog 27. Insulin was injected on the 45th day of the experiment. For eighteen days previous to the administration of the hormone, the animal had not eaten any of the deficient diet. However, no symptoms were present except for a decrease in general activity and the anorexia referred to. Gastric records taken during the ten days prior to this insulin experiment and covering a period of over fifty hours showed practically complete amotility of the stomach.

In view of the fact that insulin acts more rapidly when injected directly into the circulation, the hormone was injected into a chamber of the heart. The insulin was administered at 9:30 a.m., the same dosage being employed as in the previous experiments.

The blood sugar level was found to be 105 mgm. per 100 cc. before the hormone preparation was injected. At 10:45 a.m. there was no change in the tracing which recorded the movements of the stomach. Gastric atony was present as before. The animal appeared quiet. Blood sugar at this time was 78 mgm. per 100 cc. At 11:15 a.m. the dog became very restless although no hunger contractions developed; a blood sample was taken for estimation of its sugar content and glucose was then injected. Although the blood sugar had risen to 100 mgm. per 100 cc., the dog remained somewhat restless. This, too, suggests that insulin affects other physiologic equilibria besides that of the blood sugar level. The gastric tracing was continued for a period of six hours longer, but no significant effect of the insulin on the gastric atony was noted.

At the end of the same day, this animal developed severe symptoms; these may have been precipitated by the injection of insulin. Similar effects, the reader will recall, were observed in dog 31. Harrop (1927) demonstrated that clinically the degree of hypoglycemia does not accurately determine the severity of the insulin reaction nor does the administration of sugar always relieve it. Foshay (1927) has recently emphasized the possibility that insulin reactions are associated with cytoglycopemia, a condition in which the carbohydrate reserve within the cells is rather low. In the present study, dogs 31 and 27 manifested almost complete anorexia for the deficient diet for long periods of time. It is not inconceivable that under such conditions insulin might produce a more effective impoverishment of tissue cells with respect to carbohydrates. In any event some dogs in the advanced stages of vitamin B deficiency show what appears to be a greater sensitivity to insulin by an earlier (?) precipitous onset of neuromuscular symptoms following administration

of the hormone; the gastric motor mechanism does not readily participate in this response. Inasmuch as carbohydrate combustion supplies a great deal of the energy for muscular work, perhaps the lessened response on the part of the stomach may be attributed in part to the depleted reserve of this fuel in the muscle cells.

DISCUSSION. The results indicate that the vigorous response to insulin obtained in normal dogs is greatly diminished in those animals showing complete gastric atony incident to B-avitaminosis. In the very advanced stages of this deficiency disease gastric atony may develop to such a degree in some dogs that insulin fails to elicit any significant reaction on the part of the stomach. It is of importance in this connection to state that both of the dogs described above rapidly regained gastric tonus associated with vigorous hunger contractions after the institution of vitamin B therapy.

These results are qualitatively in accord with those previously reported (Stucky, Rose and Cowgill, 1928), although in the earlier experiments a higher incidence of positive effects on gastric atony was obtained when insulin was injected. The negative results just described may perhaps be related to the fact that the dogs used in the current investigation showed a more nearly complete gastric atony recorded for at least fifty hours on each animal before the hormone was injected.

It has previously been demonstrated (Stucky and Rose, 1929) that dogs develop anhydremia in the advanced stages of vitamin B deficiency. In view of the work of Andrews (1926) who showed that the effect of insulin is prolonged and intensified in dehydrated animals, the above findings on the effect of insulin on gastric motility would appear to be even more striking.

SUMMARY—CONCLUSIONS

The object of these experiments was to determine whether insulin would restore hunger contractions in dogs showing long-maintained gastric atony due to B-avitaminosis. Consequently, a group of dogs was fed a vitamin B-deficient ration. Two of the animals lost their hunger contractions completely and manifested prolonged atony of the stomach long before neuromuscular symptoms made their appearance. Insulin was administered to these two dogs.

An injection of the hormone in a dose which is effective in normal dogs failed to elicit a response from the gastric musculature. In one animal three successive injections of insulin resulted in a partial restoration of the hunger contractions, which, however, were very slight and associated with low gastric tonus.

Dogs in the advanced stages of B-avitaminosis seem to be peculiarly sensitive to insulin. The hormone appears to have precipitated generalized neuro-muscular symptoms in the two animals here studied. The results suggest that this heightened response to insulin is not readily shared by

the musculature of the stomach in dogs manifesting gastric atony due to B-avitaminosis.

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STUDIES IN THE PHYSIOLOGY OF VITAMINS

XII. The Effect of Parathormone on Gastric Motility in Vitamin B-Deficient and Normal Dogs[1]

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A number of investigators have demonstrated that the injection of insulin augments gastric motility (Bulatao and Carlson, 1924; Simici et al., 1927; Mulinos, 1927; Stucky, Rose and Cowgill, 1928) and raises blood calcium (Davies, Dickens, and Dodds, 1926; Brougher, 1927, and others). Smith (1927) reported that the blood calcium of B[3]-avitaminotic dogs was near the tetany level; Carlson (1916) found atony of the stomach in parathyroid tetany and the present authors showed that a marked depression of gastric motor function occurs in vitamin B deficiency (Stucky, Rose and Cowgill, 1928) resulting in complete gastric atony in some animals (Rose, Stucky and Cowgill, 1929). Other symptoms, common to a deficiency of the parathyroid hormone and of vitamin B are diminished food and water consumption, gastric stasis, vomiting, acute neuro-muscular manifestations, and lowered resistance to infection. The present communication is a report of a study of the effect of parathormone[4] on the motility of the empty stomach in normal and vitamin B-deficient dogs.

Technique. Apparently normal dogs in good nutritive state, weighing between six and fourteen kilos, were kept under observation for several weeks. The B-deficient animals were fed a mixture of isolated food substances, diet III (Cowgill, 1923), complete in every known respect except for vitamin B. Cod liver oil was administered at frequent intervals to insure an abundant supply of the fat-soluble vitamins. The normal dogs were given the same diet supplemented by an adequate amount of the vitamin B complex in the form of vitavose[5] (Hoffman, 1925).

[1] The data in this paper are taken from a dissertation submitted by William B. Rose in partial fulfilment of the requirement for the degree of Doctor of Philosophy, Yale University, 1928.

[2] Porter Fellow of the American Physiological Society, 1926–27.

[3] This term refers to the vitamin B complex (Smith, 1928).

[4] An extract of the parathyroid glands obtained through the courtesy of Eli Lilly & Company, Indianapolis, Ind.

[5] Obtained from E. R. Squibb & Sons, New York, N. Y.

The method of recording hunger contractions is described in a recent paper (Rose, Stucky and Cowgill, 1929).

Blood serum calcium was estimated by the Clark-Collip (1925) modification of the Kramer-Tisdall method. The Hagedorn and Jensen method (1923) was employed for blood sugar determinations. Hemoglobin was estimated by a modification of the Cohen and Smith technique (1919).

EXPERIMENTS. *The effect of parathormone on gastric motility in vitamin B-deficient dogs. Experiment I, dog 27.* On the 49th and 50th day of vitamin B deficiency a continuous gastric tracing extending over a period of thirty-six hours failed to show

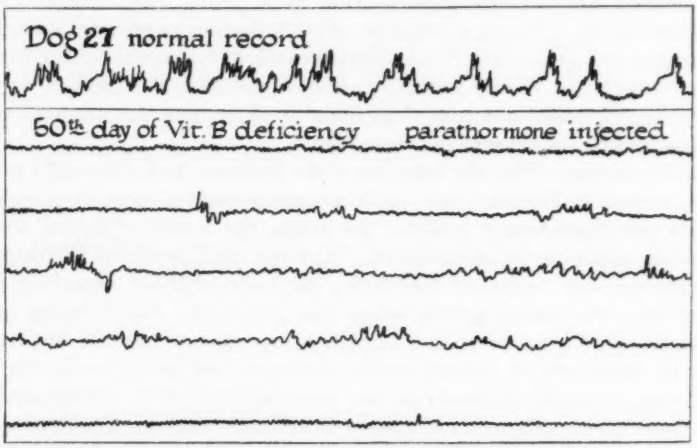


Chart 1. Dog 27, after 49 days' subsistence on a vitamin B-deficient diet, manifested prolonged gastric atony. Injection of parathormone was followed by the slight changes in gastric motility shown by the record here reproduced (approximately one-seventh original size).

a single hunger contraction. The normal variations in gastric tonus were absent. The concentration of blood sugar was normal; no symptoms, except anorexia for the deficient ration, were present. Parathormone (3 units per kilo) was injected subcutaneously. The gastric tracing was then observed for ten hours. No significant effect on gastric motility was obtained. No change in the blood sugar level or hemoglobin was noted.[6]

Experiment II, dog 27. A second experiment was performed on this dog later in the same (50th) day of vitamin B deficiency, employing five units of the hormone per kilo body weight. This time, slight variations in gastric tonus were observed. The effect is illustrated in the accompanying tracing (chart 1).

[6] A flaw detected in some of the serum calcium determinations rendered them untrustworthy—hence the values are omitted in several of these experiments.

Experiment III, dog 27. A third experiment was undertaken on this animal on the 53rd day of B-avitaminosis. Except for anorexia for the deficient diet, decrease in general activity and some loss in body weight, no abnormal symptoms were in evidence. Parathormone (3 units per kilo) was injected into a chamber of the heart. The gastric tracing was recorded for nine hours. Except for occasional aborted attempts on the part of the stomach to contract and practically negligible variations in tonus, no effect on the gastric atony was noted (chart 1). No vomiting or other symptoms were observed.

Comment. Experiments I, II and III were performed on the same animal (dog 27). The fact that a continuous thirty-six hour record failed to reveal any hunger contractions, indicates that in all probability the atony was truly complete. Hence, a negative effect following parathormone injections does not prove that this endocrine extract is incapable of producing some stimulation of the normal gastric musculature. However, subsequent experiments on normal dogs failed to show that this hormone produces any augmentation of gastric motility.

It may be argued that the influence of the hormone does not usually reach its maximum within ten hours; and inasmuch as each of these three experiments was discontinued in about ten hours, the results obtained would therefore appear to be inconclusive. However, in experiment IV (dog 31) the tracing was continued for twenty-six hours without observing any significant effect on the gastric atony that prevailed. Furthermore, inasmuch as only a few hours elapsed between experiments I and II, the latter may be considered, in a sense, a continuation of the former except for the fact that a second injection of the hormone was given. Nevertheless, nineteeen and a half hours of gastric record completed twenty-four hours after the first injection of the endocrine extract did not reveal any definite response on the part of the stomach

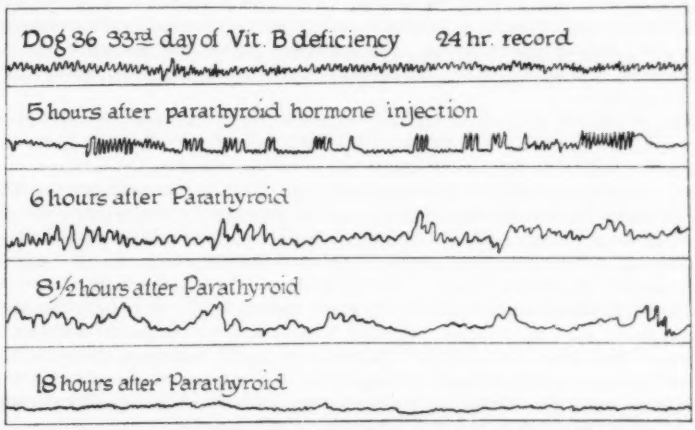
Experiment III was performed in order to determine whether the efficacy of this gland extract could be enhanced by injecting it directly into the circulation. However, the effect on gastric atony was entirely negative during the nine hours of observation. It is worthy of note that this animal was in the "advanced stages" of vitamin B deficiency but no symptoms except anorexia and a general decrease in activity were observed. Six days after experiment I (and three days after experiment III) this animal developed acute nervous manifestations.

The dog recovered after vitamin B therapy. However, the hunger contractions were still rather small ten days later, although the appetite was excellent. Nineteen days after recovery from the acute symptoms, very vigorous gastric activity was evident from the long tracings obtained.

The fact that the blood sugar and hemoglobin determinations did not reveal any significant change during these experiments appears to indicate that the quantity of hormone administered was insufficient to produce any "toxic" effect due to overdosage (Collip, 1925; 1926). Hence, the absence of a positive response cannot be attributed to this factor.

The potency of this sample of parathormone was cogently demonstrated in another series of experiments on rats (Rose and Stucky, 1929). This species, hitherto believed to be immune to the effect of a potent extract of the parathyroid glands (Collip, 1926), developed hypercalcemia[7] when injected with an adequate dosage of the same preparation used in the current investigation.

Experiment IV, dog 31. Forty-second day of vitamin B deficiency. Previous gastric tracings recorded for a total of seventy-four hours (discontinuous) during the relatively advanced stages of vitamin B deficiency showed practically complete gas-


Dog 36 33rd day of Vit. B deficiency 24 hr. record
5 hours after parathyroid hormone injection
6 hours after Parathyroid
8½ hours after Parathyroid
18 hours after Parathyroid

PARATHYROID HORMONE & GASTRIC MOTILITY IN VIT. B DEFICIENCY

Chart 2

tric atony in this dog. Except for anorexia for the deficient diet, no symptoms were present; the animal was active and lively.

First injection. Parathormone (3 units per kilo) was injected subcutaneously. Nine hours later, a slight variation in gastric tonus was observed for about ten minutes; otherwise gastric atony prevailed. However, the experiment was not discontinued at this stage.

Second injection. Fourteen hours after the first injection, a larger dose of this physiologic agent (6 units per kilo) was administered subcutaneously, and its effect on motility of the stomach recorded for twelve hours longer. The result was essentially negative. The duration of this record was twenty-six hours. At this time, no symptoms, except anorexia and two small superficial ulcers on bony prominences[8] were present.

[7] This finding was recently confirmed with parathormone (Lilly) by W. R. Tweedy and S. B. Chandler, THIS JOURNAL, 1929, lxxxviii, 754.

[8] These dermatologic lesions have been observed not infrequently in vitamin B-deficient dogs and are discussed in a recent communication (Cowgill, Stucky and Rose, 1929, Arch. Path. vii, 197).

Comment. The absence of a significant effect of two successive (14 hours apart) injections of parathormone on gastric atony during twenty-six hours of observation, renders it highly improbable that under the prevailing conditions this glandular extract is capable of stimulating gastric motility to any extent. The statements concerning the hemoglobin, sugar and calcium determinations, already made in connection with experiments I, II and III, apply here also. After vitamin B therapy was instituted the appetite returned to normal and on the ninth day of recovery excellent hunger contractions were obtained.

Experiment V, dog 36. On the 33rd day of vitamin B deficiency, a gastric tracing revealed some small hunger contractions during the early part of the experiment with some variations in gastric tonus. The quiescent period was markedly prolonged. Blood sugar—109 mgm./100 cc.; hemoglobin—116 per cent (Haldane); serum calcium 11.1 mgm./100 cc.

After a number of hours of tracing, during which no definite contractions were observed, parathormone (1½ units per kilo) was injected into a chamber of the heart. Four and a half hours later small hunger contractions were seen, but no improvement in tonus accompanied them. Approximately six hours after the hormone was administered, fairly vigorous contractions were noted associated with moderate variations in gastric tonus (serum calcium 12.1 mgm./100 cc.). One hour later vigorous hunger contractions occurred in conjunction with very distinct tonus changes and some evidence of physiologic sequence. Within one hour the hunger contractions became small, and, although tonus changes persisted, their intensity gradually diminished. Twelve hours after the hormone injection no contractions were seen, and the tonus variations were very small indeed. By the seventeenth hour even the latter disappeared entirely (serum calcium 11.0 mgm./100 cc.) and gastric atony was recorded during the remainder of this continuous twenty-four hour tracing. Short strips of representative portions of this tracing are depicted in chart 2.

Comment. At first sight, it might appear that the observed gastric response can be attributed, in part at least, to the hormone injection. While it is impossible to state that this was not the case, the authors are inclined to doubt this for the following reasons: First, all the other experiments performed on both normal and vitamin B deficient dogs fail to show that this glandular extract increases gastric motility. Secondly, some small hunger contractions were present before the hormone was administered, hence gastric atony had not yet developed. Thirdly, the dose of the hormone employed was only one-half of the smallest dose used in the other experiments and its effect on blood calcium was small (change from 11.1 to 12.1 mgm. serum calcium per 100 cc.). Fourthly, a similar twenty-four hour tracing was obtained in the case of another dog in the absence of *any* hormone administration. Thus, dog 31 (32nd day of vitamin B deficiency) revealed approximately twenty hours of gastric atony out of a total of twenty-four hours. During four hours fairly vigorous contractions were obtained. This same animal (dog 31) developed complete gastric atony five days later. Fifthly, the possibility that the hormone contained vita-

min B was considered. However, it was shown that the preparation used in these experiments does not contain any appreciable amount of this vitamin (Rose and Stucky, 1929). Finally, Collip (1925) and others have shown that the maximum effect of parathormone occurs from nine to eighteen hours after its administration. The active period observed on the tracing in dog 36 occurred between the fifth and ninth hours after the hormone was injected. In the light of these considerations, it appears highly probable that the apparently positive effect noted is without significance.

THE EFFECT OF PARATHORMONE ON GASTRIC MOTILITY IN NORMAL DOGS. The following experiments were performed on normal dogs. These animals were in excellent physical condition. Values for blood sugar, hemoglobin, total solids and blood chlorides obtained repeatedly during the observation period were well within the physiologic range. Numerous gastric tracings taken from time to time revealed good variations in tonus, normal rhythm, vigorous hunger contractions and occasional gastric tetany.

Experiment VI, dog 39. After observing a normal tracing for one hour, the hormone (15 units per kilo) was injected into a chamber of the heart. The record of gastric motility was continued for eleven hours longer. Seven hours after the record was begun the blood calcium rose 1 mgm. The hemoglobin value was practically the same as at the beginning of the experiment. Nothing worthy of note occurred. At the end of the twelfth hour the experiment was discontinued. No detectable change in gastric motility was observed.

Experiment VII, dog 40. Fifteen units per kilo of this endocrine extract were injected subcutaneously. Gastric motility was studied for a period of twelve hours. In the interim, the serum calcium increased from 11.0 to 13.0 mgm. per 100 cc. This record failed to show any significant difference from a "normal" record of the same dog. Certainly, there was no evidence of any increase in gastric motility above the normal.

Comment. These results appear to indicate that this hormone preparation administered either directly into the circulation (experiment VI) or subcutaneously (experiment VII) failed to produce any appreciable effect on gastric motility in normal dogs, within twelve hours following the injection.

In order to determine whether any significant effect developed within twenty-four hours after the parenteral administration of the same hormone, experiments VIII and IX were performed.

Experiment VIII, dog 37. A twenty-four hour tracing of the hunger contractions of this same animal was recorded one week before the administration of the parathyroid extract.

The hormone (10 units per kilo) was injected into a chamber of the heart. No significant change in blood serum calcium occurred at the end of eight hours. There was an increase of 1.1 mgm. per 100 cc. by the twelfth hour, and by the twentieth hour after the hormone injection, the serum calcium was only 0.7 mgm. above normal.

Comment. Chart 3 shows representative strips of forty-eight hours of tracing of gastric motility obtained during this experiment. An examination of the twenty-four hour record obtained after the hormone injection failed to reveal any significant deviation from the normal, when compared with a similar tracing made one week earlier. Previous gastric motility studies indicate that a degree of variability of the motor function of the stomach exists in apparently normal dogs under presumably identical experimental conditions. Therefore, minor differences in these two twenty-four hour tracings do not deserve serious consideration.

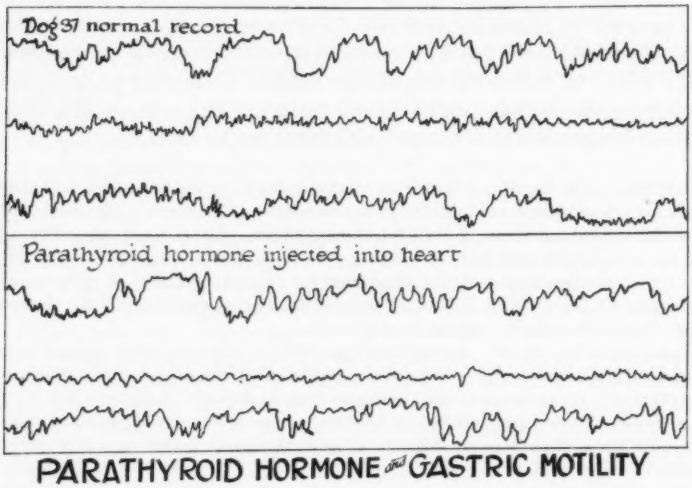


Chart 3. Reproduction of gastric tracings obtained when parathyroid hormone was injected into a normal dog (no. 37). The records here reproduced are approximately one-seventh the original size.

The relatively small increment in serum calcium observed after the hormone was administered indicates that this particular dog was apparently more resistant than other dogs to the effects of the parathyroid extract. Marked variability in the response to this hormone has been reported (Collip, 1926; Hanson, 1928). Consequently, this experiment was practically duplicated on another animal (dog 38).

Experiment IX, dog 38. After drawing blood for a serum calcium determination, parathormone (8 units per kilo) was injected subcutaneously. About five hours after the injection the dog appeared somewhat restless. This symptom recurred at intervals throughout the twenty-four hours. Nevertheless, the serum calcium was not significantly elevated during the early part of the experiment. However, the serum calcium increased markedly in the latter half of the twenty-four hour period, reach-

ing a maximum of 17.4 mgm. per 100 cc. No other untoward symptoms were evident. A continuous twenty-four hour gastric tracing was obtained.

Comment. A comparison of this twenty-four hour record with a similar tracing of the motility of the empty stomach obtained on this same animal some time prior to the hormone injection revealed a distinct *decrease* in gastric activity—especially during the latter half of the parathormone experiment.

It appears, therefore, that the administration of parathormone in sufficient quantities to produce a marked elevation of serum calcium results in a decrease of gastric motility. One factor, which may have contributed to this effect on the motility of the stomach, is suggested by the following data found in the literature. A number of workers (Collip, 1926; Cantarow et al., 1926; and others) have demonstrated the fact that excessive doses of parathormone produce a preliminary blood dilution followed by an increase in viscosity and total solid content. It has recently been shown (Rose and Stucky, 1928) that even a moderate increase in blood concentration produces a definite inhibition of gastric motor activity. Hence, the depressing effect of large doses of parathormone on the motility of the empty stomach may partly be due to the blood concentration which results.

SUMMARY—CONCLUSIONS

The effect of parenteral injections of parathormone on gastric motility in normal and vitamin B deficient dogs was studied.

This hormone produced no significant effect on gastric atony in three dogs suffering from B-avitaminosis.

Tracings of the motility of the stomach of four normal dogs were indistinguishable from similar tracings made after the hormone was injected, when the dose employed was insufficient to produce marked hypercalcemia. In one animal, depression of the motor function of the stomach occurred during the latter half of a continuous twenty-four hour gastric tracing and during this period the serum calcium was elevated over 50 per cent above the normal level.

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EFFECT OF INTRAVENOUS INJECTION OF ALKALI ON THE ACTION OF CURARE

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Past work has shown that a change in the reaction of the blood toward the acid side results in decreased excitability of the motor nerves. A change in the reaction of the blood toward the alkaline side produces an opposite effect. Slight changes in the acid base equilibrium of the tissues affect also the pharmacological action of a number of drugs. Thus Salant (1925) showed that changes in acid base equilibrium in anesthetized cats and dogs greatly affect the action of nicotine. Wenner and Blanchard (1929) found that acid decreases and alkali increases the action of strychnine. Labes (1922) reported that tadpoles are unaffected by acid solutions of atropine and cocaine while in basic solutions they die very quickly. These and other investigations suggested that the action of curare might be greatly influenced by changes in the reaction of the circulating blood.

Nineteen adult dogs were used in this investigation. Curare, a product of Burroughs, Wellcome & Company, was injected either subcutaneously or intravenously into the saphenous vein. Thirty to sixty minutes after subcutaneous injection or eight to ten minutes after intravenous injection of this drug, when paralysis of the muscles, except the respiratory muscles, had appeared, a 5 per cent solution of sodium bicarbonate was injected into the saphenous vein.

In all experiments the dogs were bled by cardiac puncture and the pH and CO_2 capacity of the blood determined. In some cases serum calcium and inorganic phosphorus also were determined.

All experimental and control animals received a lethal dose of curare. For intravenous injection the dose was 5.7 mgm. per kilo and for subcutaneous injection 8.1 mgm. per kilo of body weight.

At the onset of muscle paralysis the pH and CO_2 capacity begin to fall. Thus the pH of the blood of dog 6 (table 1) dropped from 7.4 to 7.08 thirteen minutes after receiving intravenously 59.8 mgm. of curare. The average fall, however, in our experimental animals was about pH 0.18. One animal (dog 14; table 2) showed an increase in pH and CO_2 capacity 53 minutes after a subcutaneous injection of curare. A marked degree of acidosis and anoxemia occurs as the muscles of respiration become para-

lyzed. The fall in pH evidently augments the effect of curare but is not sufficiently great in the early stages of paralysis to produce coma.

Acids greatly increase the action of curare. When curare was injected shortly after 175 cc. of $\frac{1}{15}$ molar solution of acid sodium phosphate was introduced into the blood stream, or after an acidosis had been produced by ingestion of 5 per cent solution of the acid-producing salt, NH_4Cl, death resulted within ten minutes. All controls receiving curare intravenously died of respiratory paralysis within 20 minutes. Those controls receiving curare subcutaneously died within 45 minutes.

When sodium bicarbonate is injected after a lethal dose of curare was administered recovery occurs within a few minutes. Approximately 3

TABLE 1

ANIMAL	TIME	AMOUNT CURARE GIVEN	pH	CO_2 CAPACITY	REMARKS
		mgm.		*vol. per cent*	
Dog 5 7.5 kgm.	10:42 a.m.	42.7	7.39	42	Curare injected intravenously
	10:50		7.27	40.2	Unconscious
	10:51 to 10:53				$NaHCO_3$ injected intravenously. Total 2.5 gm. $NaHCO_3$ injected
	10:55				Reflexes returned
	11:15		7.46	47.6	Normal
Dog 6 10.5 kgm.	2:30 p.m.	59.8	7.4	53.6	Curare injected intravenously
	2:43		7.08	36.5	Paralysis. Dog breathing slowly
	2:45 to 3:15				13 grams $NaHCO_3$ injected intravenously
	3:40		7.44	52.1	Complete recovery

grams of sodium bicarbonate is sufficient to inhibit the action of curare. In one case, however, 13 grams of sodium bicarbonate was injected before complete recovery resulted. The data of two experimental dogs are presented in table 1.

Burridge (1914, 1927) suggested that inhibition is essentially a decalcifying process. With calcium in excess in perfusing solutions tissues showed inhibitory phenomena less readily. In this connection the effect of calcium lactate and calcium chloride on the action of curare was studied. Table 2 contains the data of two dogs that recovered from the effects of curare after receiving approximately 7.5 grams of calcium lactate.

In two experiments in which calcium chloride was injected after curare

death resulted within ten minutes. In these experiments the increased acidity resulting from the introduction of this acid producing salt probably augmented the action of curare.

Ets (1928) reported that pilocarpine, a drug which increases muscle tone and excitability, produces a decrease in calcium in muscle tissue while atropin produces an increase in calcium. An increase in calcium according

TABLE 2

ANIMAL	TIME	AMOUNT CURARE GIVEN	pH	CO_2 CAPACITY	Ca	PO_4	REMARKS
		mgm.			*mgm. per 100 cc.*	*mgm. per 100 cc.*	
Dog 14	2:00		7.38	45.5	10.3	4.2	
21 kgm.	2:20	177.4					Curare injected subcutaneously
	3:00						Hind limbs paralyzed
	3:13		7.44	48.6	10.8	4.0	Intravenous injection of 5% solution of calcium lactate started
	3:22						Injection stopped. Dog received 175 cc. of solution
	7:30		7.34	42.0	15.5	5.8	Dog recovered
Dog 15	3:00		7.4	40.6	11.2	5.0	
13 kgm.	3:50	108					Curare injected subcutaneously
	4:15		7.34	43.0	11.0	4.3	Complete paralysis of limbs. Intravenous injection of 5% solution calcium lactate started
	4:21						Injection stopped. Dog received 150 cc. of solution
	5:40						Animal apparently normal
	6:35		7.35	45.5	18.4	5.0	

to Ets would result in depression thus favoring the action of atropin. However this may be, the results of our investigation show that the calcium content of the serum at least is not affected by curare. Large amounts of calcium injected intravenously overcome the depression and paralysis of muscles produced by curare. Salant and Washeim (1925) show that an excess of calcium decreases the depressing effect of nicotine.

SUMMARY

Alkali injected intravenously decreases the action of curare. Dogs completely recovered from the effects of curare after receiving intravenous injections of sodium bicarbonate. Acids augment the action of this drug.

At the first appearance of paralysis the pH and CO_2 capacity of the blood begin to fall. Introduction of sodium bicarbonate not only corrects anoxemia but also neutralizes the curare effect at the myoneural junction. The levels of serum calcium and phosphorus are not affected by curare.

Sufficient quantities of calcium injected intravenously also inhibit the action of curare.

The results of these experiments are in agreement with Burridge in regard to the effect of hydrogen ion concentration and calcification of the synapses upon the excitability of the nervous system.

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ELECTRICAL MEASUREMENTS OF NEUROMUSCULAR STATES DURING MENTAL ACTIVITIES[1]

I. Imagination of Movement Involving Skeletal Muscle

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It is fairly generally agreed that physiology becomes a science in so far as it expresses and measures bodily functions in the terms of physics and chemistry. But while considerable success has been attained in other departments, scarcely a beginning has been made in connection with measurements of mechanisms during mental activity. Evidently mental activity, like digestion, circulation or respiration, is a bodily function and as such is properly of interest to the physiologist; indeed, if the study of mental phenomena is ever to become a true science, it would appear to be the task of the physiologist to bridge the gap to physics. Although it is well known that some locality in the cerebrum indispensably participates during each mental activity, the possibility has not been investigated that specific change in neuromuscular tissue, varying in locality, may also occur during each mental activity.

Many investigations have been made of bodily phenomena associated with mental activity and many important conclusions have been drawn; but what takes place physiologically in the nervous or muscular system at the moment when the individual engages in mental activity has remained obscure. The nature of the relations between the mental and physiological phenomena has been the subject of much speculation in non-physiological fields, but no specific approach to this problem has been made with the methods of the physics laboratory. A brief historical review may serve to differentiate the present problem from previous studies.

In the years 1829–1834, Weber, then professor of anatomy in the University of Leipsig, published a series of studies on cutaneous and kinesthetic sensations. Following Weber's generalization from these observations, Fechner (1860), while professor of physics, added further to them and sought to deduce laws of mental measurement, that is, to develop "an exact science of the fundamental relations or relations of dependency

[1] Preliminary accounts of the present investigations have been previously published (Jacobson, 1927, 1929).

between body and mind." This science he called "Psychophysics," and it was essentially restricted to the study of sensations. The fundamental formula of this science was, in effect, that sensation is proportional to the logarithm of stimulus. The interpretations which Fechner made aroused a storm of criticism and his view that sensation can be measured in terms of the stimulus has been generally discredited. It was evidently impossible to find a scale to measure the experiences called sensations in terms of the objects or energies outside the body called stimuli. According to the present investigation, in contrast with the views of Fechner, the measurement of certain characters of mental activities might be accomplished in terms of magnitudes of specific neuromuscular changes; and, so far as I know, this possibility is here for the first time suggested and a first step taken toward carrying it out.

Among the most outstanding of subsequent attempts to link mental events to their physiological correlates were the investigations of Wundt (1902). He sought to find the physical bases of feelings mainly in terms of functions of pulse and respiration. But subsequent investigators have failed to verify such correlations. Again, Lange's (1889) observations on physiological phenomena associated with emotion have been followed by a host of other investigations, but they do not seek to attack directly the problem of the nervous or neuromuscular mechanism involved at the moment when an emotion is experienced.

Many studies have been made on the physiology of the emotions (cf. Prideaux, 1920) since the galvanometer was first adapted for that purpose (Féré, 1888; Tarchanoff, 1890; Veraguth, 1908). Generally the D'Arsonval galvanometer has been used, but occasionally the string galvanometer. During the passage of a slight constant current through the electrodes applied to the body according to the method of Féré, the occurrence of emotion is followed after a distinct latent period by a deflection. Omitting the constant current from the external circuit, Tarchanoff obtained deflections under similar conditions. The matter is reviewed by Wechsler (1925), who cites reasons for believing that the psychogalvanic reflex is due to decreased polarization in the skin, effected by sweating which immediately follows the emotion (Gildemeister, Ashley, Piéron Gregor and Loewe). Prideaux asserts (1920), apparently contrary to Gildemeister, that the skin shows decreased resistance during emotion; but both agree that the reflex is due to polarization effects. While practically all investigators agree that the psychogalvanic reflex depends upon the tissues within the skin, it is still a moot question as to what particular agent is responsible, whether sweating, or constriction of the blood-vessels in the corium (Aveling and McDowall, 1925; Densham and Wells, 1927), or changes in the epithelial cells (Richter, 1927). That sweating is at least one of the causal agents is conclusively proved in the investigations of Darrow (1927).

The results of all investigators of the psychogalvanic reaction agree that the deflection of the galvanometer follows the stimulus after a distinct interval of at least one or more seconds. It is agreed that the psychogalvanic reaction depends upon the autonomic nervous system (cf. Schilf and Schuberth, 1922; Richter, 1927), following emotion as an after-effect, or at least as a late effect or manifestation. What occurs in the nervous or neuromuscular system simultaneously with the emotion has not been investigated.

Quantitative investigations of bodily phenomena associated with mental activities, including measurements of basal metabolism, reaction time, determinations of memory curve, and plethysmographic tracings during states of attention, have been many and various, producing in many instances important results. But they do not enable us to learn what takes place in the nervous or neuromuscular system during a particular mental activity, or to measure the process in physical terms.

As early as 1912 I considered the application of the string galvanometer to the investigation of neuromuscular processes during mental activity. But this instrument as ordinarily used at a sensitivity of one centimeter per millivolt is not sufficiently sensitive for such purposes. Following the invention of the three-electrode tube (de Forest, 1907) and its further development (cf. Van Der Bijl, 1920), it was used to increase the sensitivity of the string galvanometer in physiology by Forbes and Thatcher in 1921. Since that time, others have made use of similar or modified devices in various physiological investigations, but as a rule I believed that the sensitivity fell short of the present needs. I needed to secure photographic records indicating minute oscillating potential differences continuously for periods of several minutes or more. The problem was to secure sufficient amplification, but with an instrument of such internal stability as would permit a fairly steady base-line. From the analysis of Forbes and Thatcher, it seemed best to insert a condenser in the string circuit to prevent the string from being broken by the high plate voltage. Evidently this would result in less distortion than would the use of a transformer or a modified Wheatstone bridge. On the advice of Prof. H. B. Williams, to whom I am grateful for this and other suggestions at this point, it was planned to use a string of small diameter, and upon his suggestion, I conferred repeatedly with Mr. H. E. Frederick and D. G. Blattner who designed the amplifier used. I am grateful to them for generous aid. The amplifier was donated by the Bell Telephone Laboratories, for which also I wish to express my thanks.

The aim of the experiments in the present article is to secure electrical records of neuromuscular states during specific acts of imagination. One particular act of imagination is repeated frequently with each subject. He is to "imagine bending the right arm." An imagined persistent or

steady bending of the right forearm is meant rather than an imagined brief flexion or series of alternate acts of flexion and extension. It is desired to test whether during such imagination of bending the right arm, electrical changes are present in the right biceps-brachial muscle group, and whether they are simultaneously absent from other muscular regions including the left biceps-brachial muscles. If so, this particular act of imagination, at least, would be found to be present or absent according to the presence or absence of electrical changes in the same muscles as show electrical changes during an actual performance of the act in question.

METHODS. The string galvanometer used was the Hindle electrocardiograph ("equipment no. 2"). To diminish the period of wandering

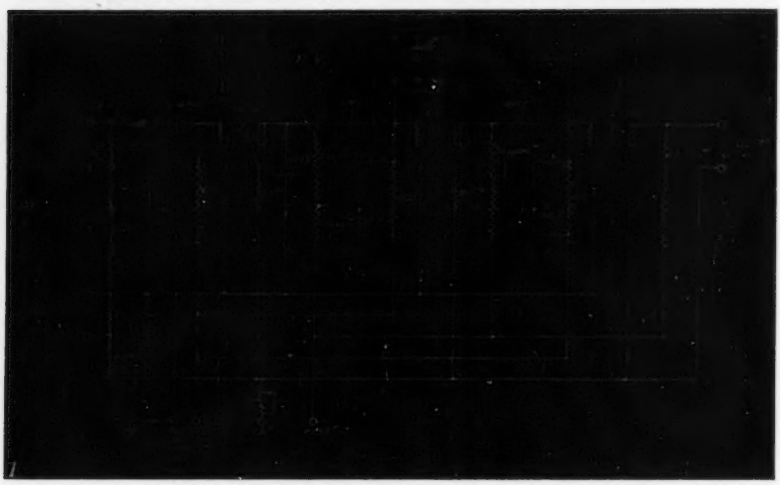

Fig. 1. The amplifier and calibration potential

of the string upon closing the circuit through the filament, a large capacity (300 MF) was inserted between the amplifier and the string (fig. 1). A variable shunt ranging up to about 50,000 ohms was placed in parallel with the string. When this dial is at zero, any potential difference in the output leads from the amplifier can not affect the string. But obviously the string will respond as the dial is turned, since the ratio of the resistance in the circuit through the string to the shunt resistance decreases. Caution is used in turning this dial lest the voltage changes from the amplifier be such as to snap the string, before their magnitude has been ascertained. In this way the shunt serves as an indispensible safeguard.

As will be evident, the amplified string galvanometer acts practically as

an electrometer; that is, deflections of the string will indicate voltage changes impressed upon the input of the amplifier, across the terminals of which is a resistance of about 155,000 ohms. Photographic records are taken of the deflections on bromide paper which moves on a coil within the camera at a pre-determined rate of speed. Horizontal lines engraved on the lens appear in the developed photographs one millimeter apart. During the course of the following calibrations as well as in the experiments when not otherwise specified, the tension of the string was adjusted so as to produce one centimeter deflection per millivolt. This is accomplished on the Hindle galvanometer by means of a standardizing battery set to supply ten microamperes of current in the string circuit when no resistance is present in the shunt of the amplifier circuit. From time to time during the course of experimentation, it is necessary to check this calibration, since the Edison storage battery, which activates the magnet, particularly if not highly charged, tends to diminish a little in amperage. By making frequent photographic records of such calibrations, corrections can be made in connection with final readings.

A more accurate method was used in the tests made with the string critically damped. In these the Edison battery was always kept fully charged so that the circuit which activates the magnet could be kept constant at 3 amperes with the aid of a suitable rheostat. Critical damping is generally necessary where precise measurements with the string galvanometer are to be made. If the string is tightened beyond a critical point, it will be found to overshoot the mark and then return to a certain level whenever a constant potential, such as one millivolt, is applied or removed. This point, at which the string just becomes damped, is determined by methods which have been described by Williams (1924, 1926). Readings from records made with a string critically damped can be corrected according to the curve and tables indicated by Williams. The string used in the present investigations was about 13.5 cm. long and 0.0018 mm. in diameter and was found to be critically damped on short circuit when the current sensitivity was approximately 1.5×10^{-6} amperes with a magnification of about 600 diameters. Deflection of the string (without amplification) was then 0.2 cm. per millivolt. Resistance of the string was 3250 ohms. Its period was in the neighborhood of 0.0011 second.

It is desired to be able to measure, from the photographs of the deflections of the string, the potential differences impressed upon the input of the galvanometer. Accordingly, the circumference of the shunt dial was marked in eleven approximately equal divisions. Evidently the amplified galvanometer system can be calibrated by impressing a known microvoltage upon the input of the amplifier and determining the corresponding deflection of the shadow in millimeters for each position of the dial.

The amplifier outfit is provided with a rheostat and a voltmeter in such

a way as to make it possible to impress upon two specific leads from the B battery one millivolt p.d. This supply was used with two Leeds and Northrup resistance boxes serving as a potentiometer arrangement to impress the desired microvoltage upon the input of the amplifier (fig. 2). Since the rheostat is set by hand so that the voltmeter reading is 4.5, it is necessary to test the degree of accuracy with which this can be accomplished. For these tests a Leeds and Northrup type K potentiometer was used, with the standard coil set at 1.0188 volts, when the sensitivity

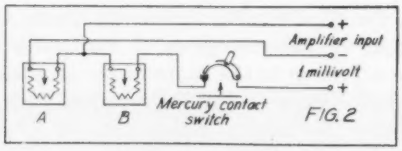

Fig. 2. Potentiometer for calibrating deflection on the photographic records in microvolts per millimeter. *A* and *B* are resistance boxes with values as shown in table 1.

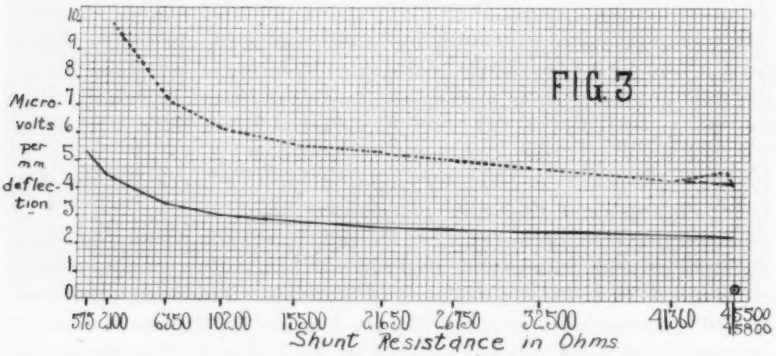

Fig. 3. Calibration of deflections (mm.) on the photographic records. Shunt resistances (abscissas) plotted against microvolts per millimeter of deflection (ordinates). Broken line: values with 75,000 ohms in the string circuit. Unbroken line: same with 25,000 ohms. Circle: zero external resistance in string circuit.

of the galvanometer was found to be 4 millimeters deflection for 10 microvolts. Room temperature was 23.3°C. Repeated tests showed that the rheostat could be set to vary but one-tenth of one per cent from the millivolt used. The error thus produced is of course negligible. Accordingly, the E.M.F. employed in the following calibrations was 0.001022 ±0.000001 volt.

The microvoltage secured from the potentiometer arrangement above described was further tested for accuracy by means of a White potenti-

ometer, wherein I had the kind coöperation of Dr. T. F. Young. An Eppley unsaturated cell is used as standard. As will be seen from table 1, these tests indicated that the potentiometer shown in figure 2 served with satisfactory accuracy to make available for calibration potential differences of 5, 10, and 20 microvolts.

Accordingly, such potential differences were impressed upon the amplifier input and photographs were taken of deflections of the string with various protective resistances in the string and the shunt circuits. For each

TABLE 1

Standardization of the calibrating potentiometer shown in figure 2

A source of 1.02 millivolts p.d. as described in the text supplies the potentiometer in figure 2. Determined values in the third column agree with those in the sixth column with the per cent of difference shown in the seventh column. Apparently the resistance selected for box B had better be 10 ohms rather than 1.

CALIBRATING POTENTIOMETER			WHITE POTENTIOMETER			PER CENT DIFFERENCE BETWEEN DETERMINATIONS (COLUMNS 3 AND 6)
Resistances		p.d. determined	Readings corresponding with column 3	Readings	p.d. determined	
A	B					
		microvolts		*For 10 microvolts*	*microvolts*	
1990	10	5.1	0.72	1.36	5.29	3.6
1990	10	5.1	0.74	1.365	5.40	5.6
1990	10	5.1	0.74	1.36	5.29	3.6
199	1	5.1	0.82	1.46	5.62	9.3
990	10	10.2	1.47	1.38	10.65	4.2
990	10	10.2	1.48	1.39	10.65	4.2
990	10	10.2	1.45	1.38	10.50	2.9
				For 20 microvolts		
490	10	20.4	2.87	2.77	20.72	1.5
490	10	20.4	2.875	2.76	20.83	2.1
490	10	20.4	2.875	2.765	20.80	1.9

combination of resistances it is then only necessary to divide the number of microvolts used by the observed deflection in millimeters in order to know the deflection in microvolts per millimeter (fig. 3). In consequence, when the two leads from the subject to the amplifier input show a potential difference (commonly called an "action current"), the value impressed upon the amplifier circuit can readily be determined.[2] The photographic records were read with the aid of a Zeiss binocular microscope, enlarging tenfold, or with a large magnifying glass.

Owing to the high sensitivity of the instrument, I was in doubt as to what type of lead-off electrodes should be attached to the subject. If the

[2]Measurements of potential differences made with this amplifier are linear.

electrodes themselves should be the source of fluctuating differences of potential, evidently the string shadow would move about, upon closing the circuit through the subject, even with his muscles completely at rest. As a result, the action potentials from whatsoever slight activity in those muscles might prove indistinguishable from the electrical effects arising in the electrodes. Early tests were made with silver wires (26 gauge) or silver plates (about 2 cm. × 2 cm.) coated with silver chloride electrolytically or else by fusion from a heated preparation of pure silver chloride. These wires or plates were inserted in a pledglet of cotton soaked in sodium chloride solution made with conductivity water. The moist cotton containing the metal could then be attached to the arms or other areas and evaporation fairly well prevented by means of adhesive tape closely applied. Solution of 0.9 per cent NaCl seemed to afford better results than did greater strength, such as 10 per cent. But the above measures failed to permit satisfactory constancy of the position of the string at higher sensitivities, and were accordingly abandoned. Several types of zinc electrodes proved even worse in this respect: *a*, zinc amalgam in the unglazed porcelain boot electrodes familiar in nerve-muscle work; the boots were filled with zinc sulphate solution, but were immersed in physiological saline externally; *b*, the zinc electrodes with kaolin described by Richter (1926), in which he apparently does not use chemically pure zinc, and *c*, small brittle plates (made by flattening ingots of pure zinc with a hammer) immersed in zinc sulphate solution. Likewise cups made of rubber or glass, with a bore of 0.5 to 1 inch, were filled with agar prepared with 0.9 per cent NaCl solution by methods familiar in physical chemistry, but a difficulty arose in making contact with the skin; for it could not be poured on when warm owing to thermal electrical effects, while after it had cooled for many hours it contracted and failed to make contact.

A series of tests was made with pyrex cells of various sizes and designs. In these were generally inserted silver electrodes said to be 99.9 per cent pure and which had been electrolytically coated in 0.9 per cent sodium chloride solution for five hours with 4 milliamperes of current. When these also failed to accomplish the desired effect, more strictly non-polarizable electrodes were made following the methods of Noyes and Ellis (1917). Platinum wire of gauge 14 or 15, after being fused into soft glass cells, was covered with a layer of freshly prepared silver oxide, which, after adequate heating at 400°C. leaves a coating of pure molecular silver. This process is several times repeated, after which the wires are coated with silver chloride electrolytically in series from 0.9 per cent solutions of sodium chloride at 6 milliamperes for 5 hours. I am indebted to Dr. T. F. Young for showing me the manner of this method as used in present investigations in the Kent Chemical Laboratory. When two such cells were filled with 0.9 per cent NaCl solution, with a yarn wick connecting

the contents of the two vessels, a test was made (without the use of a thermostat) with a Leeds and Northrup type K potentiometer. The p. d. was found to be 0.00001 volt after the vessels had been standing for a few minutes. This indicated that the electrodes were non-polarizable to the extent of meeting the requirements of what is known as "reproducible" electrodes. So far as I know, such reproducible electrodes have not heretofore been used in muscular electro-physiology.

These electrodes appeared to give better results than those previously employed, but were not yet satisfactory. Electrical disturbances still were present which I believed might be due to the electrodes or their contacts. To test whether they were due to E.M.F. arising from differences of temperature in the region of the two electrodes or their immediate connections, the arm of the subject as well as the attached electrodes was completely immersed in an oil bath kept at constant temperature (98.6°F.) with a thermometer and relay device and a motor-stirrer. In this way for the first time it became possible to secure a fairly constant position of the string, with the subject in the circuit and with the galvanometer at its highest sensitivity. But since the oil bath proved extremely inconvenient and interfered with the relaxation of the subject, an aquarian type of thermostat bath was substituted. A two-quart Ehrlenmeyer flask containing 0.9 per cent NaCl solution kept at relatively constant temperature in the bath supplied a stream of solution driven by a brass gear-pump through rubber and glass tubes to the cells. Care was taken to prevent loss of heat by means of proper insulation. But this method had to be discarded because of contamination from metal in the gears. In fact, it seemed quite necessary to keep the solution extremely free from any sort of contamination. This was accomplished by having the stream moved by suction from one Ehrlemeyer flask to another through the two cells in series. As will be seen from figure 4, the cells were supplied with side tubes and stopcocks so that, after the suction was shut off at any moment, the stopcocks could likewise be shut off. When this was done, and the tube connecting the two cells was also removed, the two electrodes were now connected in the circuit to the amplifier only through the tissues. The attempt to pass a separate stream of saline solution through each cell during an experiment had to be abandoned because this resulted in the development of potential differences apparently due to frictional electricity. Accordingly, the stopcocks were shut off as indicated at various intervals from 15 to 90 minutes of thermostatic regulation at 93° or 94°F. in this manner, and a test of the position of the string was then made to see if the experiment could be begun. In this way a greater constancy was generally secured than when without thermostatic regulation.

During the early months of investigation with the amplified galvanometer circuit, I found, like Downing, Gerard and Hill (1926), in their

experience with an extremely sensitive apparatus, that various extraneous disturbances aroused deflections of the galvanometer, making it impossible to proceed. Sparking from motors in distant parts of the building or the starting and stopping devices of a nearby automatic elevator reflected themselves in the records. In place of seeking to remove these disturbances or to shield from them by surrounding the apparatus and subject with metallic screens, I devoted most efforts to increasing what might be called the internal stability of the apparatus. In some instances where screw-connections had been made in the circuit, particularly to the electrodes, the string seemed particularly susceptible to extraneous disturbances. Accordingly, connections were made as a rule with soft solder or silver solder. Imperfections in the soldered connections frequently proved to be the source of irregular excursions of the string. Another source of instability which caused deflections resembling those from external inductance arose when the batteries became low in charge, so that it was found necessary to keep them fully charged. The principal shielding used consisted of a metal box containing the amplifier. Sometimes sheets of galvanized iron, 8×4 feet in dimensions, were placed under the couch of the subject. They did not prove indispensable, but occasionally they seemed to prevent deflections of the galvanometer occurring when the experimenter moved. For the most part it was found that the experimenter needed to remain as quiet as possible while the record was being taken. He must not, of course, touch the body of the subject, and even the act of touching the mechanical switch which governs the moving coil of the camera under some circumstances would cause string deflections. I found also that the relay system of the thermostat as well as the motor-stirrer must be shut off during an experiment, but it was possible to use the $\frac{1}{8}$ h. p. motor that comes with the camera, provided that the commutator was kept clean. An alternating current lighting circuit was previously removed from the laboratory room and direct current was substituted. Closed circuits of all sorts other than those involved in the apparatus itself were so far as possible eliminated in the room so as to minimize the likelihood of inductance effects. In this class are included lamps plugged into the direct current lighting circuit. Ground-wires were run in the usual manner to water pipes separately from the galvanometer resistance box and from the foot of the galvanometer as well as from other points indicated in figure 1. A separate ground-wire to the galvanized sheeting beneath the subject did not improve the results and was discarded.

Electrical effects possibly arising from changes in temperature were diminished by having the walls of the laboratory room prepared with Celotex building-board as well as by keeping the doors and windows closed during and previous to experimentation.

One of the most frequent sources of disturbing electrical effects evidently

came from evaporation of minute amounts of the fluid bathing the electrodes. The most effective means found to prevent this, when glass or pyrex cells are used, is by securing the cell to the limb by means of specially designed silk rubberized garters and then by squirting collodion around the external edge of the cell where it makes contact with the skin. This task needs to be carefully performed, else a minute leak may appear during the course of an experiment. A period of about ten or fifteen minutes is allowed for complete drying, after which the collodion may of itself suffice to keep the cell attached to the skin if it is desired to remove the garters. Another source of disturbing electrical effects sometimes arose from failure to place insulating material, such as cotton, between the glass stopcocks and the skin. This precaution is necessary because a thin layer of salt solution adhering to the stopcocks may act as a conductor.

In a few instances one or two punctures were made with a hypodermic needle through the skin within the site where the electrodes were to be applied, since this has been said to eliminate the skin resistance (Richter, 1927b). Since no clearly marked difference was observed in the photographs, this procedure was generally omitted.

Certain measures were taken to prevent disturbances caused by mechanical jarring of the system. The instrument was set up on the second floor, removed from the vicinity of the street and passing vehicles. Rubber sponges were placed under the amplifier box as well as under the feet of its supporting table.

When undesired disturbances of the string were eliminated in the above manner one by one in the preliminary tests, it was difficult to make certain in each case what had been the cause. On some occasions slight evaporation effects occurred along with imperfect connections or with slight chemical contaminations or with the use of electrodes which were not strictly non-polarizable; in short, a number of variables appeared to contribute to the disturbances. To have determined the importance of each of these variables in a precise manner might have been a separate investigation requiring months or even years. Accordingly, I proceeded with the practical end in view of getting the apparatus so that it would work for physiological purposes. After about a year of trials of controls of these variables, I sought from time to time to do without one or another of these controls in the hope of simplifying a technic which had become extremely cumbersome. I found that, provided other precautions as above-mentioned were taken, the non-polarizable electrodes were not indispensable.[3] In fact, similar types and magnitudes of string deflection were secured with the use of (uncoated) platinum electrodes fused in the walls of the soft glass cells. From a theoretical standpoint this is wholly comprehensible, since the registration of potential differences alternating

[3] They are preferable for records in which the electrical effects are not symmetrical with respect to the line of zero potential.

in direction frequently per second does not require non-polarizable electrodes and the action potentials of the neuromuscular effects studied had a frequency of about five to several hundred per second. Furthermore, it proved possible also to do without thermostatic regulation of the salt solution in the cells, provided that the room containing the apparatus and materials had been kept closed to exclude marked drafts for several hours before the experiment. The position of the string, while not quite so constant as before, nevertheless proved satisfactory, and a considerable saving in time with each subject was effected. Sometimes, in the aim to diminish convection currents, a little cotton was inserted in the glass cells before filling them with salt solution.

In the simplified technic of the more recent experiments, the glass cells shown in figure 5 are strapped firmly to the arm in such a way that the long axis of the attached tubes lies in the direction of the long axis of the arm. The upper cell is placed on the belly of the biceps with its center about 7 to 10 cm. above the center of the lower cell placed upon the skin in the region of the coronoid fossa.[4] Collodion is now poured around the edges of each cell and is permitted to dry. To fill the cells with saline solution, a thistle tube is used to which is attached a piece of pure rubber tubing provided with a petcock. This rubber tubing must be attached and detached from the tubing of the glass cells with great care not to disturb the collodion seal. When the free end of the rubber tubing has been attached to the proximal glass tubing of either glass cell, the subject raises his hand from the couch to a vertical or nearly vertical direction so as to facilitate the evacuation of air bubbles from the cells as the fluid is permitted to pour in through the lower end. As soon as one glass has been filled, its stopcocks are quickly turned, the rubber tubing is detached and the other cell is then filled.

Complete or almost complete relaxation on the part of the subject is required during the present physiological tests, for several reasons: 1. When the circuit is closed, including the subject, amplifier and galvanometer, with the shunt set so as to permit high sensitivity for purposes of recording minute potential differences, a sudden or a marked body-movement might snap the galvanometer string. From time to time the subject may be permitted to converse or to move, but only when the experimenter indicates to him that he may do so, because the circuit has been opened. Furthermore, if he were not generally relaxed, electromotive forces ("action currents") from active muscles would occasion deflections of the string often as great or greater than the particular deflections which it is desired to study. Such effects would displace the string, preventing the shadow from being recorded as an approximately horizontal band, just as would

[4] The upper cell thus doubtless lies below the "nervous equator" of Piper.

internal instability of the circuit or excess electromagnetic inductance. 2. Another source of disturbance might be emotions on the part of the subject, which, by arousing polarization effects in the skin (vide supra), would influence deflections of the galvanometer string. This source of disturbance likewise must be avoided by complete or nearly complete general relaxation, in which condition emotions do not take place (Jacobson, 1929, chap. 12). In order to study electrical effects arising from peripheral nerves and muscles during a particular type of mental activity, it is necessary to have a state of mental rest in the preceding and succeeding moments. This can be accomplished in persons trained by the method of progressive relaxation (Jacobson, 1925, 1929) and is found in some persons who have had no such training. An individual, with or without such training, who persisted in unabated mental activity throughout the period would obviously be unsuitable as a subject.

For these reasons most of the subjects used in the following experiment were ndividuals who had received at least several months of training to relax by methods previously described. Briefly stated, the individual receives practice to relax each of the principal muscle groups of the body in a certain order until he becomes able to relax generally to an advanced degree. Evidence of the progress of relaxation may be found in tests of the knee-jerk, which shows diminution to the stage of complete disappearance (Jacobson and Carlson, 1925; Jacobson, 1928). Repeated tests independently on 13 subjects (Jacobson, 1925) have elicited reports based upon subjective experiences that during advanced muscular relaxation mental activity is approximately in abeyance; that is to say, visual, auditory, muscular and other images present during what is commonly called thinking, reflection, attention or emotion, diminish or disappear. Certain objective reasons for believing that mental activity subsides in the presence of general relaxation have previously been recounted (Jacobson, 1929). In the instances when untrained subjects were employed, they were particularly warned to avoid sudden movements. It is also necessary to make sure that they understand that they are to imagine a persistent flexion of the forearm and not merely a very brief one.

It is agreed between subject and experimenter that upon hearing the sound of an electrical buzzer the subject shall imagine or recollect whatever is specified and that upon hearing a second similar sound, he shall relax muscularly. With suitable intervals for general relaxation, the experimenter may then give repeated pairs of signals to imagine and to relax as often as desired.

To make the signal sounds, a watchcase buzzer was operated by a dry cell with a spring key operated by hand included in the circuit. Another circuit included a dry cell or a two volt battery, a Deprez signal time marker (Cambridge Instrument Co.) and the same spring key. Accord-

ingly, the time when the buzzer sounds is indicated on the photographic record which shows the string deflections.

Various control tests (with the electrodes always on the right biceps) are made as follows: 1. To rule out the possibility that string deflections observed during imagination are due to the effects of the sound on the subject, the instruction is sometimes given for a single test occurring in a series of tests as above described, "When the next signal comes, do not bother to imagine anything; just continue to relax." If under this condition the course of the string is unaffected, it is evident that the effects of the sound are not themselves responsible for the string deflections observed in other tests. 2. To rule out the possibility that the act of imagining involves "action currents" all over the body, the subject is requested, "When the signal comes, imagine bending the left forearm." Occasionally the request is varied in the form, "When the signal comes, imagine that the right arm is perfectly relaxed." Evidently if the string's deflections are unaffected during the carrying out of these instructions, it cannot be true that the act of imagination involved the presence of "action currents" all over the body. In short, if under the above conditions positive results of a specific type are secured only during imagining which involves the use of the right arm and not during imagining which involves the use of other parts, it would follow that imagination of the type studied is a local and not a general phenomenon.

RESULTS. I. *The Character of Action Potentials from the Arm when the Subject Imagines that He is Bending it. During a period of general relaxation, when the galvanometer string with one lead attached to the right biceps is vibrating slightly and uniformly, recording a fairly steady line, the signal is given for the subject to imagine something,—for instance, to imagine that he is steadily flexing the right forearm. Generally within a fraction of a second, the string ceases its steady course and engages continually or intermittently in a less frequent series of relatively large vibrations which cease soon after the signal is given to relax any muscular tensions present* (see fig. 6). But under these conditions, if it has been agreed that the first signal is to mean "Imagine bending the left foot," the string shows no such change from small to large deflections, but generally continues unaltered in its course. This type of control test will be known as "control series 1." Similarly the string continues a fairly steady course even after signals have been given to "Imagine bending the left arm," (control series 2), "Actually bend the left foot one inch" (control series 3), "Actually bend the left arm one inch" (control series 4), "Imagine the right arm perfectly relaxed" or "Imagine the right arm paralyzed" (control series 5), "Imagine extending the right arm" (control series 6), and "When the signal comes, do not trouble to imagine anything at all" (control series 7; fig. 7).

Under favorable electrical conditions, when a photograph of the posi-

tion of the string is taken after the galvanometer magnet circuit has been closed, and with no resistance in the string circuit excepting the string itself, but with the shunt dial set at zero resistance, a straight horizontal band about 2 mm. high is seen. For convenience, a horizontal line drawn midway between the top and bottom of this band may be called the line of zero potential. If a copper wire has been placed across the input of the amplifier, acting as a short circuit, upon moderately increasing the shunt resistance, the string vibrates at a rapid rate, producing a continuous series of almost vertical lines. If now the above-mentioned wire is removed, so that the amplifier-galvanometer circuit includes the completely relaxed subject, while the shunt resistance is increased somewhat farther, the vibrations show a moderately increased amplitude but the shadow as a whole continues to be recorded as a fairly uniform horizontal band, which may be called the band during relaxation. This corresponds to what in physiology is commonly called the base line. If a line be drawn through these short vertical lines, representing their mean value, it is found under favorable electrical conditions and with advanced general relaxation of the subject to be fairly straight and horizontal and practically continuous with the line of zero potential even if high resistance has been introduced into the shunt circuit. If this line be further continued through the large vertical lines occurring during the period of imagination secured under the conditions above mentioned, it will in some records approximately bisect these lines. This means that the electrode on the biceps as compared with the other electrode passes from a state of approximately zero potential to one of negative ($-V^0_{2m}$) and then to one of positive potential ($+V^0_{2m}$) or vice versa in an alternate manner.

It may here be assumed, subject to later discussion, that the longer vibrations of the string occurring during imagination represent what are commonly known as "action currents" from nerve or muscle tissue. Ideally we might seek in the case of each line representing an "action current" to state the microvoltage value of its lowest and highest points as minus or plus from the point of zero potential. But this is not always feasible, because in many instances the entire series of vertical lines rises or falls many millimeters above or below the baseline. Under such conditions, for purposes of uniformity, it has seemed best to make the measurements of the present paper in terms of the total electrical change from instant to instant, that is, the "overall" excursion, or what is hereinafter called V^0_{2m}, the length of the vertical linear deflections expressed in microvolts. Prof. A. J. Dempster, of the department of physics, upon inquiry, kindly expressed his concurrence in this opinion.

By V^0_{2m}, then, will be meant the microvoltage value of the length of the approximately vertical lines in the photographs minus 2 mm., the width of the string shadow.

Likewise deviations up or down from the baseline or the line representing the mean value of the vertical linear deflections (that is, the line drawn through their midpoints on the photograph during relaxation) may be expressed in microvolts. The signs (+) and (−) before a microvoltage will mean respectively positive or negative potential of the electrode on the biceps as compared with the other electrode. Accordingly, V'_m will represent the value of the maximum such deviation found in any record, regardless of electrical sign.[5]

In order to count the number of vibrations of the string during imagination as compared with relaxation and to measure the amplitude of each individual vibration under these two conditions, it was found necessary to have the camera shaft revolve at an extraordinary velocity, namely, about 50 centimeters per second. When this was done with two specimen records, made with the string critically damped, the number of nearly vertical lines, representing single vibrations or semi-cycles of the string, during relaxation was found for one subject to be approximately 950 per second. These lines ranged in microvoltage (V^0_{2m}) from 0.3–9.04, with an average of 3.6. During imagination, however, many vertical lines, about 374 during each second, showed a greater magnitude; they ranged in value from 4.6 to 73.9 microvolts, and their average was 18.6 microvolts. Interspersed among these longer vertical lines occurring during imagination, there were found a certain number of short lines like those characteristic of relaxation. About 330 such lines, varying from 0.3–3.6 in microvoltage, could be counted per second. In many instances, these shorter lines occurred as projections from the larger lines in their downward or upward course, giving the appearance of ripples superimposed on waves (cf. Piper, 1912; Wachholder, 1923; Richter, 1927a).

Similar values were found for another subject. During imagination, lines ranging in microvoltage from 4.6 to 65.5 with an average of 17.7 occurred about at the rate of 423 per second. Interspersed among these also were shorter lines, like those occurring during relaxation at the rate of about 209 per second. Their range was practically the same as that of similar lines for the other subject.

These typical records, then, when subjected to complete measurements, showed vibrations of the string during imagination which represented on the average about five times the magnitude (in microvolts) of the average vibrations during complete relaxation. Intermittently among these lines, in a manner characteristic of practically all the records to be described in this paper, occurred smaller vibrations like those during relaxation. The frequency of vibrations per second was reduced during imagination about one-third.

In computing the results from the many records taken in the present

[5] Deviations, V'_m, are sometimes due to uncontrolled electrical disturbances affecting the string.

investigation, it scarcely seems necessary to measure each vertical line in a sample series in order to calculate averages, as was done in the above-mentioned two specimens. A simpler method is suggested, which would appear to have quite sufficient accuracy for the present purposes. In the tables of the present article, the microvoltages (V^0_{2m}) represented by the shortest ("min.") and longest ("max.") vertical lines occurring during relaxation are indicated. Another column contains the average microvoltage for that period, which generally lies about midway between the values for the shortest and longest lines, but which average is determined by inspection of the photographic record. This procedure seems suited to determining the approximate values for the period of relaxation, because the lines therein, under favorable conditions, are fairly uniform in length. A slightly different procedure is used for the period following the signal to imagine. Here there is considerable variation. As will be recalled, interspersed among the large vertical linear deflections commonly occur a certain number of small linear deflections of magnitudes equal to those occurring during the period of relaxation. Having stated this fact, it is evidently unnecessary to repeat it in the tables; therefore, in the column headed "V^0_{2m} During Test Periods," only the linear deflections are considered which equal or exceed the average during relaxation. The minimum and maximum of the linear deflections during test periods are determined from inspection of the photographic record and set down in the table. Midway between these two is the value indicated as the "average"; except in cases where the observed minimum and maximum values deviate considerably from the general range. In these cases, following methods commonly employed in statistics, the "average" lies midway between values determined by inspection to be fairly representative, as minimum and maximum, for the general range.

The present aim is to determine, under the conditions set up, the order of magnitude of the electrical changes led off at the electrodes following the signal to imagine. As will later be discussed, various corrections must be applied to the figures secured and eventually the procedure itself may have to be altered or refined in order to obtain precise measurements. Since critical damping is requisite for precise measurements, the results under this condition are presented first, namely, in table 2. But the general character of the results is practically like those presented in subsequent tables where critical damping was not employed. With the string in present use, there are advantages in having the tension less than that required for critical damping. A five times greater amplification can readily be secured. Furthermore, when amplification is employed, a string is in much less danger of being suddenly broken by the large deflections which occasionally occur due to unexpected electrical disturbances if it is used in a condition of lesser tension than that required for critical damping. Very prompt turning of the shunt dial is required to bring the

TABLE 2

Potential differences in electrodes on muscle during and after mental activity compared with rest (values in microvolts; string critically damped)

Under $V°_{2m}$ are the magnitudes of string vibrations as they occur during relaxation and during the time when the subject imagines that he is steadily flexing his right arm. Increase of $V°_{2m}$, indicating the presence of "action currents" from the muscle, occurs in all cases during imagination and is absent in the special control tests (see text). The absence of such increase before and after each test period ($V°_{2m}$ during relaxation) furnishes an additional type of control, which also is negative in all the tests.

Under V'_m is shown a second type of electrical change inconstantly present and frequently following the termination of the mental activity. A line through the midpoints of the vertical lines ($V°_{2m}$) on the photographs sometimes shows deviations in the form of wavelike protracted falls (−) or rises (+) in potential of the electrode on the biceps as compared with the other electrode. V'_m represents the value of the maximum deviation in each case in each record. Lesser deviations occur but are not tabulated. As will later be seen, the electrical effects classified under V'_m include the psychogalvanic reflex and are of minor interest for the present purposes.

In this and the following tables, averages are weighted in the usual statistical manner when calculating the average of averages.

RECORD NUMBER	CHARACTER OF TESTS	NUMBER OF TESTS	RESULTS OF TESTS		$V°_{2m}$						V'_m			
					During relaxation			During test periods			During or after test periods			
			+	−	Minimum	Maximum	Average	Minimum	Maximum	Average	Number of cases	Minimum	Maximum	Average
Subject B. L.														
362	Imagining	2	2	0	3	5	4	6	41	16	2	−7	−20	−13
363	Imagining	4	4	0	3	5	4	6	39	16	4	−7	−9	−8
363	Control 7	1	0	1	3	5	4	3	5	4	0			
373	Imagining	8	8	0	4	8	6	10	43	16	?			
373	Control 7	2	0	2	4	8	6	4	8	6	0			
475	Imagining	9	9	0	7	11	9	19	65	32	0			
475	Control 2	3	0	3	7	11	9	7	11	9	0			
476	Imagining	8	8	0	7	11	9	15	61	30	0			
478	Imagining	7	7	0	4	9	7	11	39	22	0			
478	Control 2	4	0	4	4	9	7	4	9	7	0			
479	Imagining	7	7	0	2	7	4	9	80	26	0			
Total imagining.....		45	45	0							6			
Total controls.....		10	0	10							0			
Average of averages for imagining.....								7		24				−10

TABLE 2—*Concluded*

RECORD NUMBER	CHARACTER OF TESTS	NUMBER OF TESTS	RESULTS OF TESTS		V^{0}_{2m}						V'_m			
					During relaxation			During test periods			During or after test periods			
			+	−	Minimum	Maximum	Average	Minimum	Maximum	Average	Number of cases	Minimum	Maximum	Average
Subject B. J.														
366	Imagining	1	1	0	4	8	6	8	20	14	1	−150	−150	−150
367	Imagining	6	6	0	2	3	2	8	20	14	4	−2	−11	−5
367	Control 7	2	0	2	2	3	2	2	3	2	0			
482	Imagining	7	7	0	2	11	9	22	98	54	0			
483	Imagining	4	4	0	2	11	9	13	65	48	0			
483	Control 2	2	0	2	2	11	9	2	11	9	0			
Total imagining		18	18	0							5			
Total controls		4	0	4							0			
Average of averages for imagining								7		37				−34
Subject B. E.														
481	Imagining	8	8	0	2	9	5	15	30	19	0			
481	Control 2	1	0	1	2	9	5	2	9	5	0			
Total imagining		8	8	0							0			
Total controls		1	0	1							0			
Average of averages for imagining								5		19				

string shadow back into the field at once. Owing to general care in this and other respects, a single string survived the entire series of present investigations covering a period of seventeen months.

Inspection of table 2 indicates that the results calculated as above described show that the large deflections (V^{0}_{2m}) following the signal to imagine are markedly increased in all cases, having a value ranging from 340 to 530 per cent of that during complete relaxation. No increase occurs in the control tests.

Following the signal to imagine, at intervals generally considerably longer than that required for the appearance of the larger linear deflections, deviations previously called "V'_m" sometimes occur. In the records of table 2, these are generally in the direction of negative potential. Their significance for physiology will be discussed later.

In table 3 are presented the results for eleven subjects following the instruction "Imagine yourself steadily flexing the right arm." To rule out the possibility that special training in observing sensations or in relaxation

TABLE 3

Same as table 2 (string not critically damped)

Increase of V°_{2m}, indicating the presence of "action currents" from the right biceps region when the subject imagines that he is steadily flexing his right arm, occurs in approximately 96 per cent of the tests; but no such increase occurs during relaxation, while the other control tests 1 to 7 (see text) likewise show negative results in approximately 93 per cent of cases.

RECORD NUMBER	CHARACTER OF TESTS	NUMBER OF TESTS	RESULTS OF TESTS		V°_{2m}						V'_m			
					During relaxation			During test periods			During or after test periods			
			+	−	Minimum	Maximum	Average	Minimum	Maximum	Average	Number of cases	Minimum	Maximum	Average
					Subject M. J.									
53a	Imagining	6	5	1	4	7	7	10	53	31	5	9	28	17
54a	Control 1	4	0	4	2	8	7	2	8	7	0			
55a	Imagining	3	3	0	2	4	3	6	23	14	3	11	14	13
56a	Control 1	3	0	3	2	4	3	2	4	3	0			
71a	Imagining	6	5	1	5	17	13	21	76	49	3	13	25	20
81	Control 2	4	0	4	11	18	13	11	18	13	0			
86	Control 2	2	1	1	16	27	20	16	30	23	1	41	41	41
Total imagining		15	13	2							11			
Total controls		13	1	12							1			
Average of averages for imagining							9			35				17
					Subject B. L.									
275	Imagining	5	5	0	0	5	3	9	55	19	5	8	29	19
275	Control 7	1	0	1	0	5	3	0	5	3	1	55	55	55
276	Imagining	4	4	0	4	6	5	9	26	17	0			
276	Control 7	1	0	1	4	6	5	5	5	5	0			
277	Control 1	5	0	5	4	6	5	5	5	5	1	8	8	8
296	Imagining	4	4	0	4	9	7	9	36	23	0			
296	Control 7	1	0	1	4	9	7	4	9	7	0			
298	Imagining	4	4	0	7	11	9	11	96	43	3	16	16	16
Total imagining		17	17	0							8			
Total controls		8	0	8							2			
Average of averages for imagining							6			25				18
					Subject I. G.									
340	Imagining	4	4	0	17	30	21	21	105	45	2	13	29	21
340	Control 7	1	0	1	17	30	21	17	30	21	0			
Total imagining		4	4	0							2			
Total controls		1	0	1							0			
Average of averages for imagining							21			45				21

TABLE 3—*Continued*

RECORD NUMBER	CHARACTER OF TEST	NUMBER OF TESTS	RESULTS OF TESTS		$V°_{2m}$						V'_m			
					During relaxation			During test periods			During or after test periods			
			+	−	Minimum	Maximum	Average	Minimum	Maximum	Average	Number of cases	Minimum	Maximum	Average
Subject D. M.														
50a	Imagining	6	6	0	9	21	17	26	139	72	6	8	126	37
50a	Control 1	4	0	4	9	21	17	9	21	17	1	80	80	80
51a	Imagining	3	3	0	9	17	13	21	76	49	3	8	21	13
6	Control 1	7	0	7	0	20	13	0	20	13	0			
8	Control 3	5	0	5	0	20	13	0	20	13	1	62	62	62
129	Imagining	4	2	2	4	18	11	11	39	25	0			
129	Control 2	2	0	2	4	18	11	4	18	11	0			
132	Control 1	3	1	2	2	7	4	2	7	4	1	21	21	21
199	Imagining	4	4	0	7	11	9	11	30	20	1	9	9	9
199	Control 2	2	0	2	7	11	9	7	11	9	0			
203	Control 6	3	0	3	7	11	9	7	11	9	0			
203	Imagining	1	1	0	7	11	9	11	32	21	0			
206	Control 4	3	0	3	4	9	7	4	9	7	0			
207	Control 5	2	0	2	4	9	7	4	9	7	0			
Total imagining.....		18	16	2							10			
Total controls.....		31	1	30							3			
Average of averages for imagining							13			43				27
Subject A. K.														
346	Imagining	2	2	0	5	9	7	13	34	24	2	8	21	15
346	Imagining	4	4	0	3	5	4	7	23	15	0			
346	Control 7	1	0	1	5	9	7	5	9	7	0			
348	Imagining	2	2	0	5	9	7	13	34	24	2	21	29	25
349	Imagining	1	1	0	0	4	1	2	34	16	0			
349	Control 1	3	0	3	0	4	1	0	4	1	0			
Total imagining.....		9	9	0							4			
Total controls.....		4	0	4							0			
Average of averages for imagining							5			19				20
Subject B. E.														
2	Control 1	4	0	4	13	20	16	13	20	16	0			
3	Control 3	4	0	4	13	20	16	13	20	16	0			
4	Imagining	5	5	0	13	20	16	23	41	32	0			
62	Imagining	8	8	0	2	3	2	3	7	5	7	3	13	8
65	Control 5	4	0	4	2	3	2	2	3	2	0			
139	Imagining	4	4	0	2	4	3	18	59	39	4	9	18	13

TABLE 3—*Continued*

RECORD NUMBER	CHARACTER OF TEST	NUMBER OF TESTS	RESULTS OF TESTS		$V°_{1m}$						V'_m			
					During relaxation			During test periods			During or after test periods			
			+	−	Minimum	Maximum	Average	Minimum	Maximum	Average	Number of cases	Minimum	Maximum	Average
Subject B. E.—*Concluded*														
139	Control 2	2	0	2	2	4	3	2	4	3	0			
140	Imagining	4	4	0	2	7	4	18	46	32	4	18	35	28
140	Control 2	2	0	2	2	7	4	2	7	4	0			
142	Control 6	4	4	0	2	4	3	4	13	9	1	28	28	28
145	Imagining	1	1	0	0	2	1	23	41	32	1	12	12	12
146	Imagining	2	2	0	2	7	4	7	39	23	0			
153	Control 1	4	0	4	4	4	4	4	4	4	0			
154	Control 2	3	1	2	4	4	4	4	4	4	1	18	18	18
320	Imagining	2	2	0	0	9	5	21	76	24	1	42	42	42
320	Imagining	1	1	0	4	9	7	9	46	27	1	60	60	60
Total imagining		27	27	0							18			
Total controls		27	5	22							2			
Average of averages for imagining							6			24				18
Subject L. G.														
11	Control 1	5	0	5	2	20	13	2	20	13	0			
12	Control 3	4	0	4	7	13	9	7	13	9	0			
13	Control 5	4	0	4	7	13	9	7	13	9	0			
14	Control 2	4	0	4	7	13	9	7	13	9	0			
15	Imagining	5	5	0	7	11	9	23	87	55	5	18	39	28
18	Control 4	5	1	4	4	13	7	16	41	28	0			
88	Imagining	6	5	1	11	16	13	18	53	35	0			
89	Control 1	3	1	2	11	16	13	11	16	13	1	21	21	21
99	Control 4	4	2	2	20	20	20	25	35	30	0			
162	Imagining	6	6	0	5	9	8	20	87	54	3	12	23	19
162	Control 2	4	0	4	5	9	8	5	9	8	0			
Total imagining		17	16	1							8			
Total controls		33	4	29							1			
Average of averages for imagining							10			48				25
Subject R. S.														
102	Imagining	4	3	1?	2	18	4	23	64	35	0			
103	Control	4	0	4	2	18	4	2	18	4	0			
Total imagining		4	3	1?							0			
Total controls		4	0	4							0			
Average of averages for imagining							4			35				

TABLE 3—*Concluded*

RECORD NUMBER	CHARACTER OF TEST	NUMBER OF TESTS	RESULTS OF TESTS		V°_{gm}						V'_m			
					During relaxation			During test periods			During or after test periods			
			+	−	Minimum	Maximum	Average	Minimum	Maximum	Average	Number of cases	Minimum	Maximum	Average
Subject H. K.														
57a	Imagining	5	5	0	11	16	13	18	36	27	5	62	62	62
58a	Control 1	5	0	5	11	16	13	11	16	13	0			
108	Imagining	5	5	0	14	20	11	16	55	35	5	60	74	68
246	Imagining	6	6	0	2	7	4	41	76	58	0			
246	Control 7	1	0	1	2	7	4	2	7	4	0			
251	Control 1	3	0	3	4	11	7	4	11	7	0			
Total imagining		16	16	0							10			
Total controls		9	0	9							0			
Average of averages for imagining							9			41				65
Subject B. R.														
112	Imagining	4	4	0	2	9	7	11	32	21	4	14	39	25
122	Control 4	4	0	4	5	9	7	5	9	7	0			
124	Control 5	3	0	3	7	11'	9	7	11	9	0			
174	Imagining	4	4	0	4	9	7	23	53	38	4	12	58	35
174	Control 2	2	0	2	4	9	7	4	9	7	0			
178	Control 6	4	0	4	7	11	9	7	11	9	0			
178	Imagining	2	2	0	7	11	9	18	41	20	2	9	12	10
179	Control 2	2	0	2	7	11	9	7	11	9	0			
Total imagining		10	10	0							10			
Total controls		15	0	15							0			
Average of averages for imagining							7			28				26
Subject B. R. J.														
125	Imagining	4	4	0	13	21	17	34	93	63	0			
125	Control 2	2	0	2	13	21	17	17	38	26	0			
196	Control 1	3	0	3	2	7	4	2	7	4	0			
Total imagining		4	4	0							0			
Total controls		5	0	5							0			
Average of averages for imagining							17			63				0

had in some manner influenced the records secured with eight trained subjects, the results from three untrained subjects (R.S., A.K., and I.G.), are likewise included in the table. As will be seen from the figures, as

well as from tables 2 and 3, the results for all subjects, both trained and untrained, are essentially alike so far as the process of imagination is concerned. During this process, marked electrical changes apparently take place in the flexor muscles of the arm whose flexion is imagined and these changes can be measured in terms of their magnitudes and durations.

In table 3 the results are positive in 96 per cent of the (141) tests during the imagination and negative in 93 per cent of the (149) control tests 1 to 7. They are negative also before and after each period of imagination, which furnishes us with an additional type of control test.

The signal to relax muscularly is followed by the subsidence of the large linear deflections for all the trained subjects in practically all instances and as a rule for the untrained subjects, with exceptions to be noted below. These few exceptions evidently depended upon failure of the untrained subject to relax. All subjects agreed that imagination ceased following the signal to relax and all agreed that the imagination of contracting the forearm had as an essential component an experience as of extremely slight muscular tenseness in the biceps region. The finding that it is apparently impossible to have a motor imagination of bending the right arm during complete relaxation of the right arm (Jacobson, 1925) is confirmed. Sometimes visualizing the right arm in flexion also was present in the imagination, but discussion of this matter is postponed to a later article.

It will be recalled that during the period of training to relax, prior to the present investigations, the subject is never instructed to "try to cease thinking," or "make the mind a blank" but only to relax muscles. Accordingly, following mental activity, when the string returns to the shorter vibrations and the relatively quiet course characteristic of the conditions of complete relaxation, the report subsequently secured from the subject is to the effect that no direct effort or attempt to cease thinking was made: all subjects agreed that the signal to relax muscles meant the same to them as if the instruction had been to cease to imagine and they had successfully done so. From the subjective viewpoint, mental activity subsides as the muscles become extremely relaxed. The instruction to relax in this manner was given repeatedly if the subject did not appear to be relaxed as judged by visible slight restlessness, speaking, and an irregular baseline. Apparently, when the instruction was effective, it was followed by the fairly uniform short vibrations occurring in a fairly horizontal line which in the present article is called the line during relaxation.

II. *Reaction Times, Relaxation Times, and Duration of Electrical Effects.* Table 4. *For most of the subjects (6 out of 11) in most of their tests (56 out of 63) the period from the sound of the buzzer to the initiation of the long vibrations of the string (V^0_{2m}) varies from about 0.2 to 0.6 (± 0.1) second.* This range applies likewise to the other subjects, excepting one, in about half of the instances. The exceptional subject has longer reaction times

TABLE 4

Reaction times, relaxation times and duration of electrical effects for imagination

Reaction times are the intervals from the beginning of the buzzer signal to imagine to the onset of the long linear deflections, V°_{2m} (column B), or to the beginning of deviation of curve, V'_m (column D). All times are stated in seconds.

The duration of "action currents" shown in column F obviously depends directly upon the interval between the signal to imagine and the signal to cease to imagine. But the duration of deviations, V'_m, shown in column H, does not depend upon this interval, for V'_m frequently begins long after the first signal, and sometimes even after the second. Furthermore, V'_m is often absent altogether, as will be seen from column G. The times shown in column J are calculated from the beginning of the second buzzer sound to the point when the long linear deflections first show a clear-cut diminution. "Times to complete disapnearance" are reckoned from the beginning of the buzzer signal to cease imagining to the end of long deflections, V°_{2m} (column L), or to the end of deviations V'_m, (column N).

That the deviations of curve V'_m commonly arise considerably later than the "action currents," V°_{2m}, is shown in column O; and that the former, when present, commonly considerably outlast the latter is shown in column P.

RECORD NUMBER	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
	Reaction times				Durations				Time to initial diminution of "action currents" V°_{2m}		Time to complete disappearance					
	To "action currents" V°_{2m}		To V'_m		Of "action currents" V°_{2m}		Of V'_m				Of "action currents" V°_{2m}		Of V'_m		D–B	N–L
	Number of tests	Average time	Number of cases	Average time	Number of tests	Average time	Number of cases	Average time	Number of tests	Average time	Number of tests	Average time	Number of cases	Average time		
53a	5	2.7	5	6.5	5	6.0	5	6.8	0		5	−0.9	5	4.2	3.8	5.1
55a	3	3.5	3	3.7	2	1.8	2	4.7	0		1	0.4	2	3.6	0.2	3.2
71a	4	1.5	3	1.1	5	3.4	4	2.4	4	0.4	4	0.8	3	0.8	−0.4	0.8
86	0		1	0.6	0		1	3.0	0		0		1	−1.0		
Total....	12		12		12		12		4		10		11			
Average of averages....		2.5		3.9		4.2		4.7		0.4		0.7		3.1	1.4	2.4
275	5	0.4	5	1.0	5	2.1	6	2.4	5	0.2	5	0.2	6	1.3	0.6	1.1
276	4	0.5	0		4	2.0	0		4	0.2	4	0.2	0			
296	4	0.5	0		4	2.1	0		4	0.2	4	0.2	0			
298	4	0.5	3	0.9	4	3.4	3	3.6	4	0.5	4	0.7	3	1.4	0.4	0.7
Total....	17		8		17		9		17		17		9			
Average of averages....		0.5		1.0		2.4		2.8		0.3		0.3		1.3	0.5	1.0

TABLE 4—*Continued*

RECORD NUMBER	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
	Reaction times				Durations				Time to initial diminution of "action currents" V°_{2m}		Time to complete disappearance					
	To "action currents" V°_{2m}		To V'_m		Of "action currents" V°_{2m}		Of V'_m				Of "action currents" V°_{2m}		Of V'_m		D-B	N-L
	Number of tests	Average time	Number of cases	Average time	Number of tests	Average time	Number of cases	Average time	Number of tests	Average time	Number of tests	Average time	Number of cases	Average time		
340	3	0.5	2	5.2	4	3.1	2	2.6	4	0.4	4	0.7	2	0.8	4.7	0.1
Total....	3		2		4		2		4		4		2			
Average of averages....		0.5		5.2		3.1		2.6		0.4		0.7		0.8	4.7	0.1
50a	4	0.5	5	2.0	5	3.7	5	7.7	3	−0.5	3	−0.3	5	6.1	1.5	6.4
51a	3	0.4	3	2.5	3	6.1	3	7.8	2	0.5	2	4.9	3	6.0	2.1	1.1
8	0		1	1.3	0		1	9.4	0		0		1	6.7		
129	1	0.2	0		2	2.6	0		1	0.4	1	0.6	0			
132	0		1	2.5	0		0		0		0		0			
199	4	0.3	1	1.6	4	3.2	1	0.6	3	0.2	3	0.4	1	1.1	1.3	0.7
203	1	0.3	0		1	2.8	0		1	1.9	0		1	3.9		
Total....	13		11		15		10		10		9		11			
Average of averages....		0.4		2.1		3.8		7.2		0.6		1.9		5.5	1.7	3.6
346	6	0.6	2	0.5	6	2.6	2	6.0	6	0.4	6	0.5	2	3.7	−0.1	3.2
349	1	0.5	0		1	1.9	0		1	0.2	1	0.4	0			
348	2	0.5	2	1.6	2	4.6	2	0.8	2	0.7	2	2.0	2	−0.8	1.1	−2.8
Total....	9		4		9		4		9		9		4			
Average of averages....		0.6		1.1		3.0		3.4		0.4		0.8		3.7	0.5	2.9
4	5	2.2	0		5	4.5	0		1	1.0	3	0.8	0			
62	6	1.1	7	3.9	6	4.0	6	5.7	6	0.3	7	0.4	5	4.4	2.8	4.0
139	4	0.4	3	0.5	4	1.7	3	2.2	3	0.1	3	0.2	4	0.9	0.1	0.7
140	4	0.4	4	0.7	4	1.6	4	2.6	3	0.1	4	0.1	4	1.4	0.3	1.3
142	4	0.5	1	0.4	3	2.7	1	2.4	3	0.8	3	0.8	0		−0.1	−0.8
145	1	0.5	1	0.9	1	2.5	1	2.2	1	0.2	1	0.6	1	0.7	0.4	0.1
146	2	2.2	0		2	6.0	0		2	0.2	2	0.2	0			
320	3	0.8	2	0.9	3	4.5	2	5.5	3	0.2	3	0.3	2	0.9	0.1	0.6
Total....	29		18		28		17		22		26		16			
Average of averages....		1.0		1.9		3.4		3.9		0.4		0.4		2.1	0.9	1.7

TABLE 4—*Concluded*

RECORD NUMBER	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
	Reaction times				Durations				Time to initial diminution of "action currents" V°_{2m}		Time to complete disappearance					
	To "action currents" V°_{2m}		To V'_m		Of "action currents" V°_{2m}		Of V'_m				Of "action currents" V°_{2m}		Of V'_m		D–B	N–L
	Number of tests	Average time	Number of cases	Average time	Number of tests	Average time	Number of cases	Average time	Number of tests	Average time	Number of tests	Average time	Number of cases	Average time		
15	5	0.3	5	0.5	5	3.2	5	4.0	5	0.3	5	0.4	5	1.1	0.2	0.7
88	5	1.1	0		5	3.3	0		4	0.5	4	0.8	0			
89	0		1	2.0	0		1	3.4	0		0		1	0.7		
162	6	0.4	2	1.3	6	2.8	2	1.8	6	0.3	6	0.5	3	0.9	0.9	0.4
Total....	16		8		16		8		15		15		9			
Average of averages....		0.6		0.9		3.1		3.4		0.4		0.5		1.0	0.3	0.5
102	2	6.0	0		2	219+	0		3	0.8	2	210+	0			
Total....	2		0		2		0		3		2		0			
Average of averages....		6.0				219+				0.8		210+				
57a	4	1.7	4	1.8	5	1.2	5	10.6	5	−1.7	5	−1.6	5	7.8	0.1	9.4
108	5	1.3	5	2.1	5	1.8	5	8.2	2	0.8	2	0.9	5	5.9	0.8	5.0
246	4	0.7	0		6	1.7	0		3	0.1	3	0.1	0			
Total....	13		9		16		10		13		12		10			
Average of averages....		1.2		2.0		1.6		9.4		0.6		1.0		6.9	0.8	5.9
112	3	0.3	4	0.4	4	2.0	4	2.1	2	0.2	2	0.2	2	0.5	0.1	0.3
174	3	0.2	3	0.3	4	1.8	4	3.4	3	0.6	3	0.7	4	1.3	0.1	0.6
Total....	6		7		8		8		5		5		6			
Average of averages....		0.3		0.4		1.9		2.8		0.4		0.5		1.0	0.1	0.5
125	4'	0.7	0		4	5.5	0		4	1.0	4	2.5	0			
Total....	4		0		4		0		4		4		0			
Average of averages....		0.7				5.5				1.0		2.5				

as a rule. On the whole the above-mentioned reaction times are longer than those generally reported from psychological laboratories as occurring when the subject presses a key as rapidly as possible following a prearranged stimulus. This is quite to be expected, since in the present tests

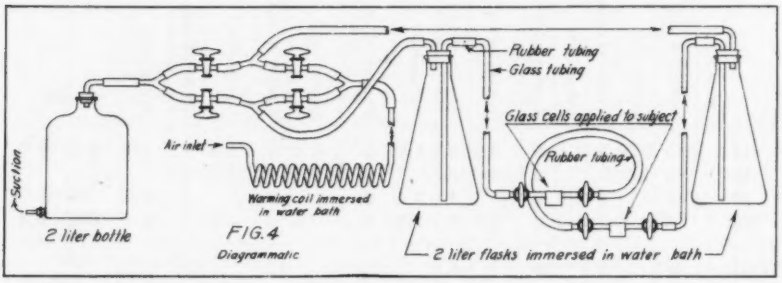

Fig. 4. Diagram of apparatus to equalize temperature of saline solution in glass cells applied to subject. The two liter flasks are immersed in a water bath containing a de Kotinsky mercury contact relay for thermostatic regulation. After the cells have been sealed on the arm of the subject, the fluid can be sucked alternately from cell to cell until an equal temperature has been attained. Both cells and all tubing are heavily covered with cotton to protect against outside air-currents. Upon closing the glass stopcocks, air currents were sometimes introduced beneath the cotton. To avoid this, in later experiments the glass stopcocks were not used, but metal clamp-cocks were substituted which could be operated outside the cotton by means of five inch rods which projected. Closing a cock, even tightly, does not always break an electrical connection, because of the presence of a thin layer of connecting fluid. Therefore, joints are made in the rubber tubing by cutting and inserting a short glass tube; so that after the cocks are closed, the joints between the cells and between the cells and the two-liter flasks can be conveniently broken.

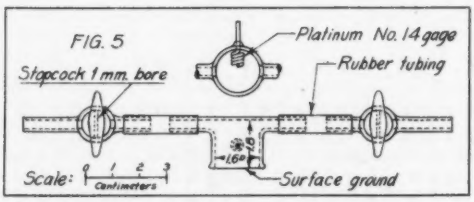

Fig. 5. Glass cells applied to arm of subject, making a fluid contact

no instruction is given to the subject to react as rapidly as possible. Furthermore, in certain unpublished tests on association, I have found the reaction time markedly prolonged during advanced general relaxation as compared with conditions when relaxation is not attempted. This also harmonizes with the present finding of a somewhat prolonged reaction time.

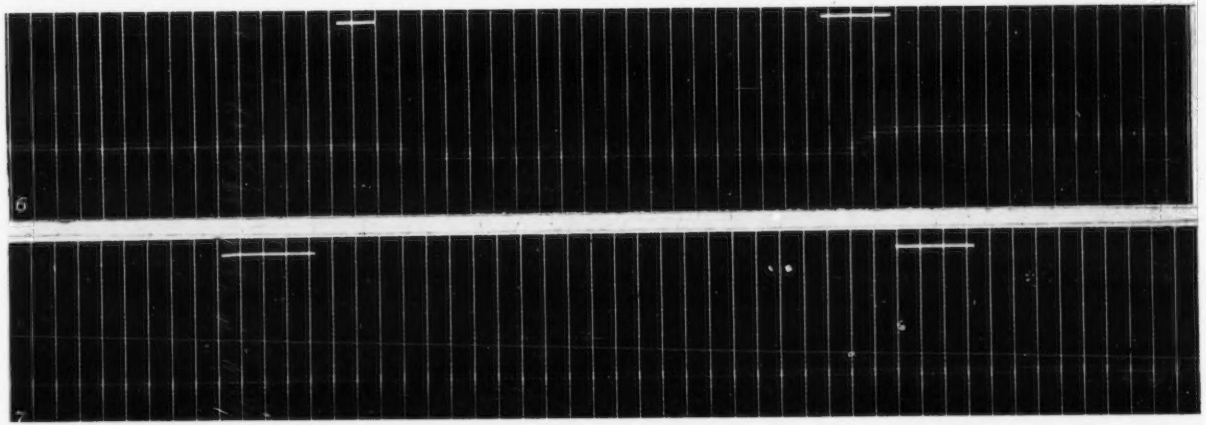

Fig. 6. Photographic record of string deflections during imagination (subj. B. L.). The subject imagines that he is bending his right arm, while one electrode is over the right biceps region and the other is over the coronoid fossa. Upper line: first break indicates the signal to "imagine bending the right arm;" second break indicates the signal to relax muscularly. Before the first signal is the period of relaxation, in which the string is seen to be relatively quiet. Soon after the beginning of the first signal (here 0.4 second), the string shows long linear deflections, which cease shortly after the beginning of the second signal. Downward deflections indicate negative potential of the electrode on the biceps compared with the other electrode. Distance between parallel horizontal lines is 1 mm. = 2.3×10^{-6} volts. Vertical markings indicate time in $\frac{1}{5}$ second. Magnification about 600. String critically damped. Other characteristics described in text. Record no. 357

Fig. 7. Photographic record of control test (subj. B. L.). Conditions same as for figure 6. The electrodes remain connected with the right biceps region, but the instruction has been, "When the signal sounds, do not bother to imagine anything at all." Under these conditions, no electrical change in the biceps follows the first signal. Record no. 357.

Prolonged rises or falls of curve (V'_m) occur in somewhat more than half the total number of tests with all subjects, 81 out of 142. Of these 81 instances (subtracting 5 uncertain cases) half begin from 1 to 9.6 seconds after the beginning of the signal, while the other half begin less than 1 second after the beginning of the first signal. But they are not evenly distributed in the time intervals under 1 second, since most of them (21 out of 38 instances) occur from 0.2 to 0.5 second, inclusive, after the beginning of the first signal.

The duration of the prolonged rises or falls of curve ranges as a rule from 1.2 to 6 seconds. It bears no apparent relationship to the duration of the large deflections (V^0_{2m}) which is determined by the signals to imagine and to relax.

As will be seen from table 4, the rise or fall of curve, when present, lags behind the onset of the large deflections by a considerable interval; but the former generally outlasts the latter by a still greater interval.

In the present study, marked increase in the magnitude of the string vibrations (V^0_{2m}) has been found to be characteristic of practically all the instances following the signal to imagine flexion of the respective arm. Inspection of the photographic records in figures 6, 8, and 9 also brings out this point clearly. On the other hand, rises or falls of curve (V'_m) appear in the photographs as highly variable phenomena and, as said before, are absent in about half the instances. The interpretation of these rises and falls of curve may be more conveniently considered below, following the discussion of the linear deflections.

The coincidence of the occurrence of imagination and of lengthened string vibrations is clearly brought out in certain additional tests. A signal magnet was arranged to record on the photographic record a line which could be broken when the subject pressed the button of a switch with his left index finger. He was instructed to press the button during the period when imagination took place, releasing it at other times. After a little practice, this could be successfully done. To be sure, a perfect parallelism in time between the large linear deflections and the indicated process of imagination is not expected. Deviations of a fraction of a second will doubtless occur, since this period of time may be required for the subject to perceive that he is imagining or has ceased to imagine. In order to gain some idea of the reaction time of these three subjects, as they lay upon the couch with eyes closed, they were requested to press the button during periods when a buzzer sounded. For all three subjects the average value was 0.24 second.

According to the signal line thus made by the subjects in these tests, the period during which imagination takes place coincides with the period during which the string shows lengthened vibrations except for fractions of a second at the beginning or end. As anticipated, the reaction time of

the subject in pressing the button needs to be taken into account. Fifty figures were secured for the three subjects, in total, representing the discrepancies in onset or conclusion between the period of lengthened vibrations and the period of imagining indicated by the subject. The average for these figures was found to be practically identical with their average reaction times as determined by the method described in the previous paragraph,—namely, 0.23 second. The extremely close correspondence of the latter figure to the value 0.24 previously mentioned is probably in part accidental; but making due allowance for this, the result indicates that the period of imagining coincides with the period of lengthened vibrations, subject to the slight discrepancies introduced by the subject's reaction time.

Following the onset of the second signal, which signifies that the subject is to cease to imagine, there generally occurs with subjects who have been trained to relax a distinct diminution of the large deflections within a time interval of 0.2 to 0.5 second. A complete disappearance of such deflections generally occurs under the same conditions within 1.5 seconds. Exceptions to this generality were infrequent with the trained subjects, but they occurred more often with two out of three of the untrained subjects. Most notably, R. S., untrained, failed to relax as indicated by the persistence of lengthened deflections, on one occasion for seven minutes and on another occasion for more than two.

In almost all instances, under our conditions, any rise or fall of curve, if present, vanishes on the average within four seconds after the disappearance of the large linear deflections. In sum, if we define relaxation-time as the time from the onset of the second signal to the point where the curve no longer shows large linear deflections or other marked deviations, we may say that under our conditions it generally was complete within 5.5 seconds.

III. *Comparisons with Actual Flexions of Right Forearm.* In order to aid the interpretation of the above results concerning imagination, further tests (about 53) were conducted with six of the same subjects. The instruction was to bend the right arm actually, but as slightly as possible. No mechanical records of these movements were taken, but the experimenter was able as a rule to discern a movement of the hand of apparently about one centimeter or somewhat more. Actual arm movement would have sent the shadow off the field if the circuit had been set at the same sensitivity as was used for the tests on imagination. Therefore a higher resistance was inserted in the string circuit and a lower resistance in the shunt circuit.

Under these conditions of lower sensitivity of the galvanometer, photographic records of slight actual flexion of the right arm are in general appearance practically the same as those shown for imaginary flexion.

Data for such actual flexions are summarized in table 5. In microvoltage the action potentials exceed those during imagination by percentages ranging from 33 for the lowest subject to 410 for the highest.

For purposes of comparison with the time relations shown in table 4, similar data for these slight actual movements are presented in table 6. Here the reaction times to the beginning of the lengthened deflections, as

TABLE 5

Potential differences in electrodes on muscle during contractions following the instruction to perform a very slight actual flexion

The contents of this table are to be compared with those of table 3. For the two tables, the ratio of the sum total of the averages of average values of $V°_{2m}$ (column 11) for same subjects is 33 microvolts/76 microvolts. That is, the microvoltage is about doubled in a slight actual movement as compared with imagining. In table 5 deviations V'_m occur in 35 out of 52 tests, which is about 10 per cent more often than in table 3. The average microvoltage for V'_m for all subjects is approximately 55 for table 5 compared with 26 for table 3. Here again the microvoltage is doubled for slight actual movement as compared with imagining.

RECORD NUMBER	CHARACTER OF TEST	NUMBER OF TESTS	RESULTS OF TESTS		$V°_{2m}$						V'_m			
					During relaxation			During test periods			During or after test periods			
			+	−	Minimum	Maximum	Average	Minimum	Maximum	Average	Number of cases	Minimum	Maximum	Average
Subject L. G.														
19	Bend right arm	2	2	0	4	13	7	46	64	55	2	58	67	62
98	Bend right arm	4	4	0	20	20	20	50	140	95	1	140	140	140
100	Bend right arm	1	1	0	10	10	10	20	70	45	0			
101	Bend right arm	1	1	0	9	9	9	34	68	51	0			
Total number		8	8	0							3			
Average of averages							14			73				101
Subject H. K.														
59a	Bend right arm	1	1	0	10	10	10	40	110	75	1	40	40	40
59a	Bend right arm	1	1	0	9	9	9	31	87	54	1	44	44	44
59a	Bend right arm	1	1	0	10	10	10	19	88	54	1	50	50	50
59a	Bend right arm	1	1	0	11	11	11	15	90	53	1	50	50	50
59a	Bend right arm	1	1	0	12	12	12	13	89	51	1	40	40	40
59a	Bend right arm	1	1	0	13	13	13	16	71	43	1	62	62	62
110	Bend right arm	4	4	0	10	30	15	20	140	80	4	50	160	98
Total number		10	10	0							10			
Average of averages							13			65				55

TABLE 5—*Concluded*

RECORD NUMBER	CHARACTER OF TEST	NUMBER OF TESTS	RESULTS OF TESTS		V''_{2m}						V''_m			
					During relaxation			During test periods			During or after test periods			
			+	−	Minimum	Maximum	Average	Minimum	Maximum	Average	Number of cases	Minimum	Maximum	Average
					Subject B. E.									
64	Bend right arm	1	1	0	12	12	12	24	84	54	0			
64	Bend right arm	4	4	0	12	12	12	20	70	45	1	90	90	90
148	Bend right arm	2	2	0	5	10	8	30	150	90	0			
148	Bend right arm	2	2	0	5	10	8	30	180	105	2	30	30	30
149	Bend right arm	2	2	0	2	4	3	18	53	35	0			
154	Bend right arm	1	1	0	4	4	4	64	87	76	1	69	69	69
159	Bend right arm	6	6	0	7	7	7	9	110	87	6	16	46	30
Total number		18	18	0							10			
Average of averages							8			72				55
					Subject M. J.									
75	Bend right arm	5	5	0	26	26	26	113	176	144	5	75	100	93
Total number		5	5	0							5			
Average of averages							26			144				93
					Subject B. R.									
115	Bend right arm	2	2	0	2	10	6	10	20	15	2	10	10	10
120	Bend right arm	5	5	0	5	9	7	17	93	55	5	17	25	19
Total number		7	7	0							7			
Average of averages							7			44				15
					Subject I. G.									
344	Bend right arm	4	4	0	5	5	5	40	80	60	0			
Total number		4	4	0							0			
Average of averages							5			60				0

in the former tests, mostly lie between 0.2–0.6 second (33 out of 53). Prolonged rises or falls of curve again occur in somewhat more than half of the total number of tests, 31 out of 53. Of these 31 instances, about one-third (12) begin from 1 to 4.3 seconds, inclusive, after the beginning of the signal, while the other two-thirds (20) begin less than 1 second after the beginning of the first signal.

TABLE 6

Reaction times, relaxation times and duration of electrical effects for contractions following the instruction to perform a very slight flexion

The contents of this table are to be compared with those of table 4. As will be seen, the time characteristics of "action currents" during imagination and during the intentional performance of slight actual movement are practically identical.

RECORD NUMBER	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
	Reaction times				Durations				Time to initial diminution of "action currents" V°_{2m}		Time to complete disappearance					
	To "action currents" V°_{2m}		To V'_m		Of "action currents" V°_{2m}		Of V'_m				Of "action currents" V°_{2m}		Of V'_m			
	Number of tests	Average time	Number of tests	Average time	Number of tests	Average time	Number of cases	Average time	Number of cases	Average time	Number of cases	Average time	Number of cases	Average time	D-B	N-L
19	1	0.2	2	0.3	2	1.7	2	2.4	2	0.8	2	0.8	2	1.7	0.1	0.9
98	4	0.5	1	2.4	4	2.5	1	2.5	4	0.2	4	0.7	1	1.7	1.9	1.0
100	1	0.7	0		1	4.5			1	0.3	1	0.3				
101	1	0.5	0		1	4.0			1	0.2	1	0.2				
Total....	7		3		8		3		8		8		3			
Average of averages...		0.5		1.0		2.7		2.6		0.4		0.6		1.7	0.5	1.1
59a	5	0.7	6	0.9	6	1.2	6	3.0	4	1.7	4	1.8	4	1.1	0.2	−0.7
110	4	0.9	4	2.2	4	1.6	4	2.4	2	−1.3	1	0.1	3	2.3	1.3	2.2
Total....	9		10		10		10		6		5		7			
Average of averages...		0.8		1.4		1.4		2.8		1.7		1.5		1.6	0.6	0.1
64	5	0.9	1	3.2	5	4.2	1	4.6	5	0.2	5	0.3	1	3.3	2.3	3.0
148	4	0.4	2	1.5	4	2.7	2	1.1	4	0.7	4	0.7	2	0.7	1.1	0
149	2	0.4	0		2	2.5			2	0.4	2	0.7				
154	1	0.2	1	0.6	1	1.3	1	1.8	1	0.6	1	1.4	1	1.3	0.4	−0.1
159	4	0.4	4	2.0	4	4.2	4	3.9	4	0.5	4	1.3	4	2.9	1.6	1.6
Total....	16		8		16		8		16		16		8			
Average of averages...		0.5		1.9		3.4		3.0		0.5		0.8		2.2	1.4	1.4
75	5	0.4	5	0.5	5	2.2	5	4.0	5	0.5	5	0.6	5	2.5	0.1	1.9
Total....	5		5		5		5		5		5		5			
Average of averages...		0.4		0.5		2.2		4.0		0.5		0.6		2.5	0.1	1.9

TABLE 6—*Concluded*

RECORD NUMBER	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
	Reaction times				Durations				Time to initial diminution of "action currents" V°_{2m}		Time to complete disappearance					
	To "action currents" V°_{2m}		To V'_m		Of "action currents" V°_{2m}		Of V'_m				Of "action currents" V°_{2m}		Of V'_m			
	Number of tests	Average time	Number of tests	Average time	Number of tests	Average time	Number of cases	Average time	Number of cases	Average time	Number of cases	Average time	Number of cases	Average time	D–B	N–L
115	1	0.4	1	0.6	2	3.6	1	1.4	1	0.1	1	0.2			0 2	
120	4	0.2	4	0.4	4	1.1	4	1.4	2	0.1	2	0.4	4	0.3	0 2	−0.1
Total....	5		5		6		5		3		3		4			
Average of averages...		0.2		0.4		1.9		1.4		0.1		0.3		0.3	0.2	0
344	4	0.5	1	0.5	4	1.8			4	0.1	4	0.3				
Total....	4		1		4				4		4					
Average of averages...		0.5		0.5		1.8				0.1		0.3				
350	4	0.3	0		4	2.3			4	0.1	4	0.6				
Total....	4		0		4				4		4					
Average of averages...		0.3				2.3				0.1		0.6				

The duration of the rises or falls of curve (V'_m) is about the same as in the tests on imagination; but the duration of such deviations seems to vary somewhat with the duration of the lengthened vibrations (V^0_{2m}) in table 5, which did not appear to be true in table 3. As in the tests of imagination, the rise or fall of curve, when present, lags behind the onset of the lengthened vibrations by a noteworthy interval. And again, the former deviations generally outlast the latter vibrations.

Accordingly, marked lengthening of vibrations (commonly called "action currents") is found to be characteristic of all the instances of muscular contraction, like those of imagination of contraction. But rises or falls of curve, V'_m, appear in the photographs as highly variable phenomena, and are frequently absent. What has been said for the disappearance times of the lengthened vibrations during imagination in table 3 holds, with minor differences, for the similar data in table 5. The principal difference is that relaxation is complete on the average in a shorter period in the case of the actual movements, namely, in 3 seconds or less, as compared with 5.5 seconds or less.

All subjects agreed that the experience of muscular tension in the present series of tests of slight actually bending the right arm was identical with the experience during imagined bending of the same part excepting that the former was much more intense.

IV. *Correction of Errors in Measurements.* We have not in the above investigations made our measurements at the probable source of the potential differences, namely, in the individual muscle fibre or nerve fibre itself. Consequently, there occurs to an unknown degree a shunting of electrical effects through other tissues and fluid. It may some day be possible to estimate the magnitude of this error at its source. With this accomplished, intensity of efferent or afferent discharges as they occur in the tissues might be correlated to microvoltage of deflection. The attempt to do this must await further experiments. But under the present conditions, our measurements serve to indicate the potential differences *as they occur at the recording electrodes.*

Errors to be considered are chiefly as follows: 1. Corrections of the readings of the values of the linear deflections can be made, if the string is critically damped, according to methods and tables presented by Williams (1924). It is then necessary to know the frequency of the impressed voltage. 2. Slight errors, of course, occur in reading the lengths of the large vertical deflections. 3. Slight errors arise in the application of statistical methods to the measurements above described. Correction of the above three types of errors has not seemed of consequence in the present article. 4. Upon changing the external resistance in the string circuit from 25,000 ohms to zero, lower readings in microvoltages of action potentials result, sometimes amounting to 50 per cent. Much or all of this discrepancy could probably be avoided by taking all measurements with the string critically damped and making corrections accordingly.

According to the investigations of Adrian and Bronk (1929), the action potentials from a single muscle fibre, under given conditions, have a practically constant voltage. If so, a record made from a single muscle fibre during imagination would reveal a certain frequency of such constant values and no further measure would thereby be obtained. Obviously it is valuable for purposes like the present to obtain a photographic record of something more than the mere presence or absence of a constant potential in a given fibre; a record is desired which to some extent represents the sum total or integration of the responses of various fibres as tested with the given set of electrodes. This involves the difficulty that some fibres are responding out of phase with others, and it is difficult to find a single expression for the sum total of the magnitude of responses. To do this, we must await the development of methods which will effectively indicate the proportion of fibres which participate in a given contraction of a muscle bundle.

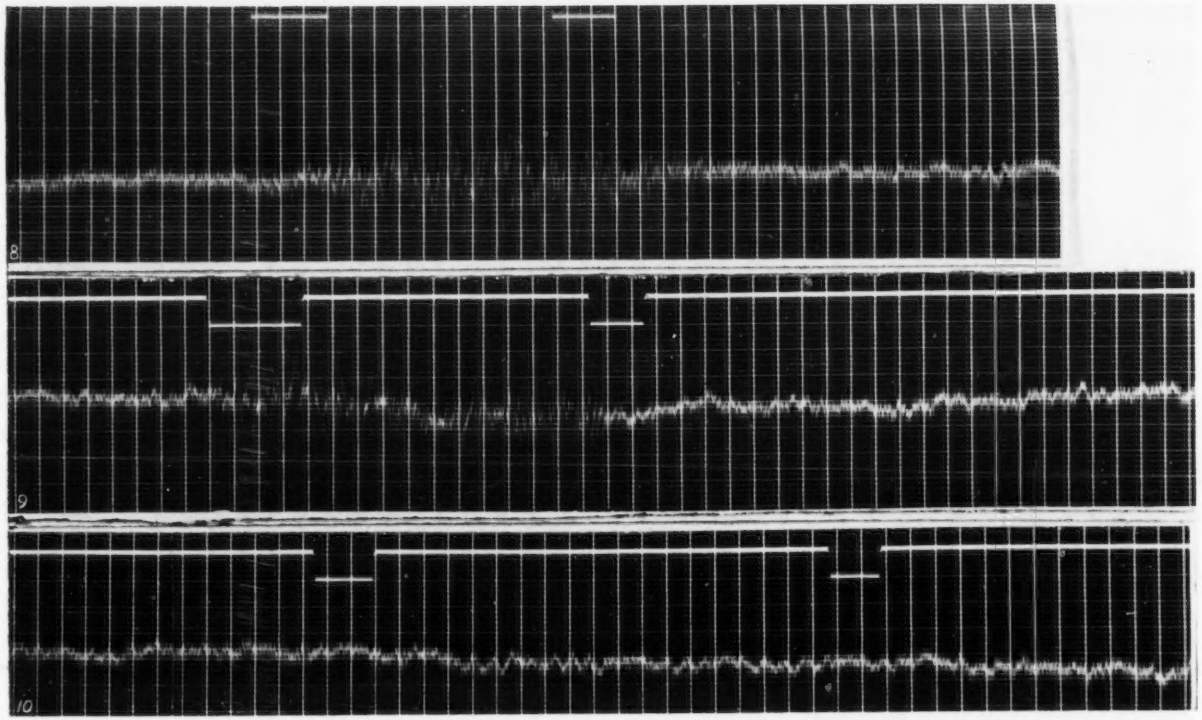

Fig. 8. Photograph of string deflections during imagination (subj. I. G.). Conditions and instructions same as for figure 6, except that string is not critically damped; 1 mm. = 4.2×10^{-6} volts. Record no. 340.

Fig. 9. Photographic record of string deflections during imagination (subj D. M.). Conditions same as for figure 8. For instructions, see under figure 6; 1 mm. = 2.3×10^{-6} volts. Record no. 199.

Fig. 10. Photograph of control test (subj. D. M.). The electrodes are connected the same as for figure 9. Upper signal line: first break indicates the signal to "imagine bending the left arm;" second break indicates the signal to relax muscularly. Under conditions otherwise the same as for figure 9, no electrical change in the right biceps is recorded following the first signal. Record no. 199.

DISCUSSION. Under the conditions described in the foregoing pages, lengthened deflections of the string were observed during imagination of flexing the arm to which the leads were attached. These deflections indicated fluctuations in potential, occurring frequently per second,[6] and it is now in order to discuss from what tissue in the region of the biceps they arise, whether muscular, nervous, dermal or vascular. Inspection of the photographic records taken during imagination and of those taken during slight muscular contraction indicates their identity in all essential respects except magnitude of microvoltage. Comparisons of the tabulated results for these two types of activity shows a similar parallelism of characteristics. Furthermore, various investigators following Piper (1912) have published records of "action currents" from human muscles during voluntary activity which essentially resemble the various photographs presented in the present studies (cf. Forbes and Thatcher, 1920; Wachholder, 1923; Richter, 1927a).

However, it might be conceivable that the action potentials recorded under the present conditions arise from the peripheral motor nerves alone. But results secured by other investigators indicate that when action potentials like those under discussion are present in peripheral motor nerves, the muscle tissue responds at a corresponding rate (Dittler and Garten, 1912; Gasser and Newcomer, 1921). Even if the contention of certain earlier investigators were assumed to be correct that muscle tissue is not always able to follow the most rapid rates of discharge in the nervous system, there would be no implicit denial that the muscle tissue responds in the present instances, although at a slower rate, and that its action potentials are being recorded, alone or with those from the nerve tissue. Evidence from the work of previous investigators as well as from the present investigations at any rate leaves no doubt that the action potentials during the present investigation arise from neuromuscular tissue.

Granting the presence of action potentials in certain muscles during imagination of voluntary contraction the question arises whether they necessarily mean that actual contraction of some fibers is present, thus arousing a faint muscular sensation on the part of the subject. I am inclined to believe that this occurs. Most of the above subjects, as well as about fifty others, have had their attention specially directed toward their muscle sense during a prolonged period of training (Jacobson, 1929), resulting in an apparently increased ability to recognize and localize sensations from muscular contraction. According to reports repeatedly secured in daily tests for the past seven years, the subjects have an experience as of muscular tension somewhere with every conscious activity

[6] It is important to note that Davis and Brunswick (1926) have concluded that "a valid objective measure of the intensity of the nerve impulse is the E.M.F. (either maximum or average) of the action current."

observed. For instance, upon imagining that they are flexing the right forearm but not actually performing this act, or upon imagining a ten pound weight being lifted in the right hand, they have generally reported an experience as of slight tension in the biceps at the moment of imagining, but disappearing when imagination ceased. In the present investigation this matter is put to objective test. We find in the instance examined that the motor type of imagination is present or absent with the presence or absence of electrical signs of contraction in the flexor muscles. Complete relaxation of the flexor muscles, producing an absence of action potentials and proprioceptive sensations, does away with the motor imagination. This leaves open the question whether in the absence of electrical signs of contraction in a part, the individual can still have a visual picture of the part in contraction or can say to himself that the part is contracting. The data gathered in the present studies would be entirely consistent with an affirmative answer to the above-mentioned question.

That vascular tissues are not responsible for the rapid and large variations in potential (V^0_{2m}) here reported is indicated in records made with electrodes placed directly over blood vessels which in one subject were conspicuous under the skin. Rises and falls of curve were recorded somewhat like the T wave in the normal electrocardiogram, but nothing like rapidly oscillating potentials.

The character of rises or falls of the mean value line of the curve has been described above and values V'_m are indicated in tables 2 and 3. In general such deviations were found to fall into two classes,—about half of them beginning from 1 to 9.6 seconds after the beginning of the signal to imagine. These deviations are apparently identical in their general characters with what has by many previous investigators been called the psychogalvanic reflex. It should be emphasized that the period of lengthened vibrations, found to be characteristic of all the mental activity in the present study, differs from psychogalvanic reflex phenomena in two essential respects: 1. The string vibrations occur at varying rates from five to several hundred per second (this higher rate doubtless arising when the nerve-muscle responses are not all in phase), while the psychogalvanic reflex shows no such frequency per second, but is a single rise or fall of curve which commonly lasts considerably more than a second. 2. The electrical changes here found characteristic of imagination of motion follow the stimulus after a brief time interval, averaging 0.4 second in about two-thirds of all the present tests. This feature alone would serve to distinguish them sharply from psychogalvanic phenomena, which according to all previous investigators begin 1 to 4 seconds after the stimulus. In agreement with the present point of view, Wells and Forbes (1911) likewise emphasize that the psychogalvanic reflex has a relatively long latent interval, about

three seconds in their experiments, and therefore is to be distinguished from "action currents" due to contractions of voluntary muscles.

In certain of the present records which cannot be reproduced because of insufficient space, what is evidently the psychogalvanic reflex is seen following the first signal after an interval of one or more seconds. While emotion is for the most part absent during the present experiments and particularly with the subjects best trained to relax, exceptions occur, as when at first the subject is as yet unaccustomed to the tests, or when the subject is awakened from sleep by the buzzer signal and experiences a feeling of being startled, or when distress arises from the constriction caused by the rubber arm-bands. Under such conditions, rises and falls of curve, evidently the psychogalvanic reflex, are common occurrences, and for the most part they act as hindrances to securing the steady base-line which is most desirable for recording the large deflections characteristic of imagination.

No final conclusion can be drawn as to the origin of those rises and falls of the curve (V'_m) which occur less than one second after the beginning of the signal to imagine motion. Possibly in some instances they are polarization effects in the skin or in some way dependent upon changes of resistance in the skin.[7] Considering the close relationship in time, although somewhat delayed in their onset, with the large deflections shown in actual muscular contractions (tables 5 and 6), they remind us somewhat of the "tonus curve" of Ewald (1910), Fröhlich and Meyer (1912), and Schäffer (1927). But this resemblance may well be merely superficial, and the electrophysiology of the so-called "tonus curve" is as yet an unsettled matter.

SUMMARY AND CONCLUSIONS

The present conclusions relate to one simple act of imagination of flexion of the forearm which has been frequently repeated in order to secure statistics of measurements.

1. When the subject, lying relaxed with eyes closed, engaged in imagining that he was steadily bending his right forearm, action potentials were led off from a suitable electrode placed over the right biceps, while a second electrode led off from a relatively indifferent point on the right arm.

2. No such potentials were recorded under the same conditions, including the same signal sounds, when the subject continued to relax and did not engage in imagination. Likewise the results were negative when the subject engaged in imagining that he was performing acts with other parts of his body, such as bending the left foot or bending the left arm, or when he actually bent the left foot or the left arm very slightly.

3. Evidence is presented that the records secured during imagination are identical in type with records secured when the subject has been in-

[7] See also footnote on page 582.

structed to make slight actual muscular contraction, excepting only that the microvoltage is considerably less in the former instances.

4. It is therefore concluded that the total physiological activity present when there is imagination of voluntary movement includes neuromuscular processes in the locale comprised in the imaginary act.

5. A method of objectively measuring these neuromuscular processes is presented, and the possibility of the development of a branch of study analogous with physical chemistry, and which may appropriately be called physical psychology, is herewith opened.

6. The action potentials that occur during imagination of motion are very different from the psychogalvanic reflex.

7. Relaxation of the neuromuscular processes characteristic of the occurrence of mental activities is generally accomplished within 1.5 seconds in the present experiments, when the motor imagination disappears. This is a confirmatory instance of the previous finding that diminution of mental activities can be brought about by muscular relaxation (Jacobson, 1925, 1929).

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ON THE TOXICITY OF PURIFIED BILE PREPARATIONS

II. The Neuro-Muscular System

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In a previous paper one of us (1929) reported on the toxic properties of chemically pure bile acids and bilirubin. In that study it was observed that the intravenous injection of bile salts caused marked circulatory depression and eventually failure. Often an animal would show muscular twitching and spasms. Still and Carlson (1929) observed that the intravenous injection of bile caused muscular twitching, retching or spasms in unanesthetized dogs. Clinicians have long recognized some of these symptoms as well as paresthesia as part of the jaundice complex. Meltzer and Salant (1904) found that injection of bile into frogs produced two results: coma and tetanus, the former result being the more frequent. Macht and his co-workers (1923–24) reported that the injection of small doses of bile salts in white rats depressed the higher centres as manifested by maze running studies.

In this study we have noted the effects of purified bile salts on the neuromuscular system, in an attempt to localize, if possible, the effect and site of action of these salts upon the system. The bile preparations used were samples prepared by one of us, as described previously (Still, 1929).

1. Effects of injection of bile salts on the behavior of frogs. When a large dose of a bile salt (1.0 mgm. sod. cholate per gram) is injected into the ventral lymph sac there usually ensues within 2 minutes a period of hyperexcitability characterized by twitching of the muscles and motor hyper-activity. The average duration of this period is 10 minutes. Following this period, when the animal is placed in water, it is unable to swim or does so in a sluggish and incoördinated manner. Then there is difficulty in regaining the normal position when placed on the back and in 35 minutes the frog cannot right itself. The ability to swim disappears; if the frog is strongly stimulated while in water the movements are feeble and incoördinated. It no longer squats but shows paralysis and loss of posture, the extremities usually remaining in the position in which they are placed. The response to mechanical stimulation disappears unless the stimulus is intense. The respiration and circulation become weaker and finally cease (50 per cent in 2 hours, 50 per cent in 3 hours).

With the smaller doses (0.25 mgm. sod. cholate per gram) the period of hyperactivity persists as long as 35 minutes, to be followed by a period of sluggish response to mechanical stimulation and a loss of posture. Swimming is coördinated but slow. Within 6 hours the animal is usually apparently normal and remains so.

It was noted that the lethal dose was somewhat larger than reported in the first paper in this series. This may be due to the fact that in the former work winter frogs (poor condition) were used, whereas spring frogs were used in the experiments reported in this paper.

Experiments upon decerebrate frogs give a similar picture.

2. Action of bile salts on frog's muscle-nerve. Attempts were made to study the effect of bile salts on isolated muscle-nerve preparations suspended in oxygenated Ringer's solution by adding the bile salt to the Ringer's solution. Since the rate of diffusion, etc., could not be controlled the results were variable and not easily interpreted.

a. Injection experiments. Quantitative measurements were made in the following manner. The pithed frog was attached to a frog board suspended above a muscle lever. The sciatic nerve was exposed and cut (or left intact) and placed on silver shield electrodes. The gastrocnemius muscle was dissected out, preserving the blood supply, and the lower tendon attached to the muscle lever. The control on each experiment was to determine the threshold stimulus of the nerve and the stimulus for maximal contraction, as measured by the response of the muscle. A coreless coil was used which contained, in the primary circuit, a large variable resistance. One of the various bile salts was then injected into the ventral lymph sac (0.06 to 2.0 mgm. per gram). At five minute intervals the threshold and stimulus for maximal contraction were again measured. The rate and force of the heart beat were noted between each measurement (direct inspection).

In case of very small doses (0.08 mgm. sod. cholate per gram) the preparation became hyper-irritable and remained so for 1 to 3 hours, and in some experiments for 10 hours after injection. If the injection did not cause circulatory failure and the nerve was not damaged the preparation had usually returned to the normal response 24 hours after injection. Within five minutes after the injection of a slightly larger dose of bile salt (0.12 mgm. sod. cholate per gram) the muscles often showed marked twitching and occasionally general extensor contractions with an increase in the irritability.

With still larger amounts (as 0.5 mgm. sodium cholate per gram) there was a decrease in irritability from the start. The changes in threshold and current required for maximal contraction in one such experiment are seen in figure 1-A. After a depression period of 30 to 45 minutes there is usually a period of hyperexcitability. This hyper-irritable state may

persist as long as an hour, generally giving rise to depression. The changes occurring in the amount of current for maximal contraction are always more marked than changes in the threshold and at the end of the experiment the difference between the two strengths of current may be 2 to 3 times that of the control period. With still larger amounts (1.0 to 2.0 mgm. per gram) the depression was more marked and progressive from the onset and the circulation and respiration stopped in 1 to 1.5 hours. The height of the maximal contraction remained practically unchanged and when the nerve showed complete block the muscle responded effectually to direct stimulation.

The action of sodium choleate and desoxycholate was very similar to that observed in the foregoing, except that their toxicity was more marked. The

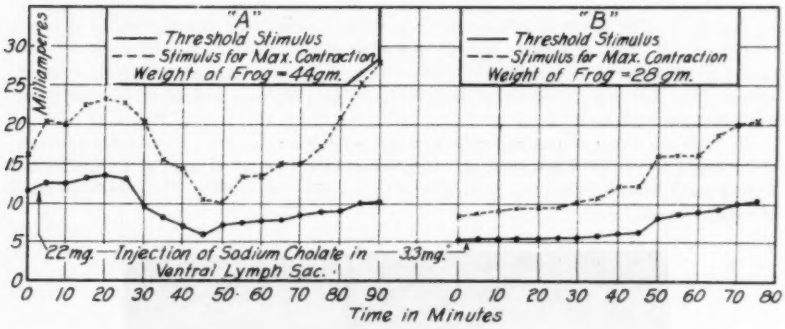

Fig. 1. *A*—Effect of injection of 0.5 mgm. of Na-cholate per gram. *B*—Effect of injection of 0.12 mgm. Na-cholate per gram.

dose used was correspondingly diminished. An experiment showing progressive depression is shown in figure 1-B. As our method of determining circulatory efficiency was not quantitative we are unable to say to what extent the changes in irritability can be correlated with changes in the circulation.

These data seemed to show that very small doses of the various bile salts increase the irritability of the muscle nerve preparation; slightly larger doses decrease the irritability, this being followed by an increase and then a depression; still larger amounts primarily cause depression without a period of increased irritability. We do not believe it possible to associate quantitatively the dosage with the effects noted because of the absence of 1, accurate data on the circulation; 2, accurate data on detoxifying agents, such as sterols, etc., and 3, accurate data on the amount of salt actually reaching the parts studied. Therefore perfusion studies were carried out.

b. Perfusion experiments. The perfusion studies were carried out on

frogs using Jackson's technic, with the procedure of registration previously described. The frog was perfused through the aorta. Figure 2A shows one such experiment. The threshold of the preparation was 15 m. a. (A), the stimulus employed was 20 m. a. at one minute intervals. Eleven control contractions were obtained (B) and then 16 mgm. sodium choleate (54

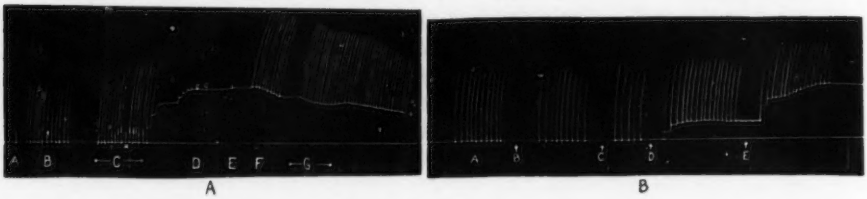

Fig. 2. A—Perfusion of a 54-gram frog with Na-cholate. *A*—threshold of nerve = 15 m.a.; *B*—controls using 20 m.a. stimulus; *C*—perfusion with 16 mgm. of Na-cholate; *D*—loss of irritability of nerve; *E*—electrodes introduced into muscle, threshold = 100 m.a.; *F*—control contractions using 600 m.a. stimulus; *G*—perfusion with 16 mgm. of Na-cholate.

B—Perfusion of a 53 gram-frog (curarized) with Na-cholate. *A*—control contractions using stimulus of 600 m.a.; *B*—Perfusion with 2 cc. of Ringer's; *C*—perfusion with 5 mgm. Na-cholate; *D*—perfusion with 5 mgm. Na-cholate; *E*—perfusion with 10 mgm. Na-cholate.

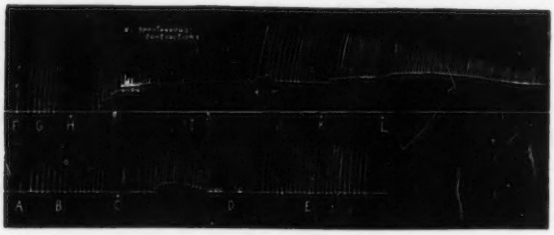

Fig. 3. Perfusion of a 50-gram frog with Na-cholate. *A*—threshold = 26.1 m.a.; *B*—controls using stimulus of 30 m.a.; *C*—perfusion with 10 mgm. Na-cholate; D—no response using stimulus of 30 m.a.; *E*—stimulus of 135 m.a.; *F*—Threshold more normal = 60 m.a.; *G*—stimulation with 135 m.a.; *H*—perfusion with 5 mgm. Na-cholate; *I*—complete loss of irritability of nerve; *J*—electrodes in muscle, stimulus = 600 m.a.; *K*—perfusion with 5 mgm. Na-cholate; *L*—perfusion with 2½ mgm. Na-cholate.

gram frog) in 2 cc. of Ringer's were perfused in 14 minutes (C). During this period there was an increase in the height of the contractions, followed by contracture, a few feeble contractions and then complete loss of irritability of the nerve, even to the strongest stimulus (D). Silver wire electrodes were introduced into the muscle; the threshold was found to be at 100 m. a. (E) the stimlus employed 600 m. a. Control contractions were made (F);

as will be noted these were as high as those obtained previously in the hyperirritable state following perfusion. Subsequent perfusion (G) of 16 mgm. sodium choleate (14 minutes) gave a gradual decrease in the height of the contraction and a tendency to return to the base line. Figure 3 shows an experiment in which small doses of sodium cholate were perfused and demonstrates the spontaneous muscle twitches, progressive decrease in the irritability of the nerve and also the fact that the muscle is affected by bile salts to a slight degree.

TABLE 1

The effect of bile salt (Na) on oxygen consumption of sciatic nerve fibers

The brackets indicate nerves from the same frog

MENSTRUM	BILE SALT CONCENTRATION	TEMPERATURE	OXYGEN CONSUMPTION	REMARKS
	mgm. per cent	*°C.*	*mm³/gram/hour*	
Ringers	0	20.5	28.0	
Ringers + Choleate	125	20.5	25.5	
Ringers	0	20.5	31	Oxygen consumption constant during 9 hours
Ringers + Desoxycholate	125	20.5	27	
Ringers	0	20.5	25	
Ringers + Cholate	125	20.5	22	
Ringers	0	21.5	38	
Ringers + Choleate	25	21.5	39	
Ringers + Desoxycholate	25	21.5	38	
Ringers + Desoxycholate	2.5	21.5	35	Oxygen consumption constant during 10 hours
Ringers + Cholate	2.5	21.5	40	
Ringers + Choleate	2.5	21.5	37	
Ringers	0	21.5	38	
Ringers + Cholate	25	21.5	37	

c. Curarized preparations. In order to study the action of the bile salts upon the muscle only, a curare effect was produced by the method described by Kleitman and Crawford (1924). Both injection and perfusion experiments demonstrated that bile salts have a toxic action on the muscle fibres, but not as marked as on nerve. Contracture and primary hyperexcitability were noted in these experiments as in the intact muscle-nerve preparation, but in no case did we note spontaneous muscle twitches. When amounts less than 0.5 mgm. per gram were injected in the ventral lymph sac in some instances there was hyperirritability; with still larger doses

depression. Figure 2-B shows the effect of perfusion of 20 mgm. of sodium cholate in broken doses.

d. Nerve fibre. The nerve fibre was affected only by exposure to very strong solution (1 per cent) of the bile salts; washing with Ringer's usually restored conductivity to the nerve fibre.

We were interested to know the effect of bile salts on oxygen consumption of nerve fibers. Sciatic nerves of green frogs were removed and placed in either Ringer's buffered with phosphate to pH 7.7 or in buffered Ringer's containing bile salts. The volume of fluid used was 0.4 cc. in each case. The nerves were then placed in Warburg's manometers and the oxygen consumption followed for 8 to 10 hours. Table 1 shows the results obtained in the experiment. These data show that small concentrations of bile salts have no effect on oxygen consumption of nerve fibers but that high concentrations (125 mgm. per cent) lower the consumption by 10 to 12 per cent.

3. THE EFFECT OF BILE SALTS ON THE CROSSED REFLEX INTO THE GASTROCNEMIUS MUSCLE. *a. Perfusion experiments.* An hour was allowed after the decerebration for recovery from spinal shock. The technic of the perfusion was similar to that of the experiments on muscle-nerve. The vessels of the right leg were ligated close to the pelvis and sectioned. In two experiments the vessels were ligated in the pelvis. Tracings were made of the contraction of the right gastrocnemius muscle. A mercury key was attached to the writing lever, so that when the muscle contracted the circuit was broken. The sciatic nerve of the left side was stimulated. In this phase of the work we employed a standard Harvard inductorium, using tentanizing shocks of constant intensity. Silver wire shield electrodes were used; the strength of the stimulating current was kept constant, and was not of sufficient intensity to injure the nerve.

The results of the perfusion experiments varied according to the concentration and kind of bile salt employed and the rate of the perfusion. In one such experiment (fig. 4-A) 6 mgm. of sodium cholate in 1 cc. of Ringer's solution were perfused during a period of 13 minutes. There was a progressive decrease in the height of the muscle contraction obtained, associated with a marked increase in the time of stimulation necessary to cause reflex contraction (increase in latent period) passing on to complete block. Then 1 cc. of Ringer's solution was rather rapidly perfused (1 minute). The preparation then showed hyperirritability: an increase in the height of the contraction with a decrease below the control reading of the time of stimulation necessary for contraction (decreased latent period). A subsequent perfusion (3 minutes later) of 2 mgm. of sodium cholate caused complete block from which the animal did not recover on perfusion with Ringer's solution. At this time the muscles of the homolateral side showed but feeble contraction on stimulation. In the experiment shown in figure 4-B perfusion of 2.4 mgm. of sodium cholate in 2 cc. of Ringer's solution over a

period of 45 minutes resulted in a progressive increase in irritability (shortened latent period), followed by depression and block; perfusion of 2 cc. of Ringer's solution resulted in a hyperirritable state. Subsequent perfusion of 5.8 mgm. of the cholate (over a period of 55 minutes) in 3 cc. of Ringer's solution gave a repetition of the results with a block from which there was no recovery. In no other experiment was so large an amount of sodium cholate necessary to produce the results shown.

b. Injection experiments. The technique was similar to that described in the perfusion experiments, except that the salt was injected into the ventral lymph sac. The injection of sodium cholate in the dose of 0.5 to 1.0 mgm. per gram weight brought on a period of hyperexcitability with spontaneous muscle twitches, followed within 5 minutes by a gradual

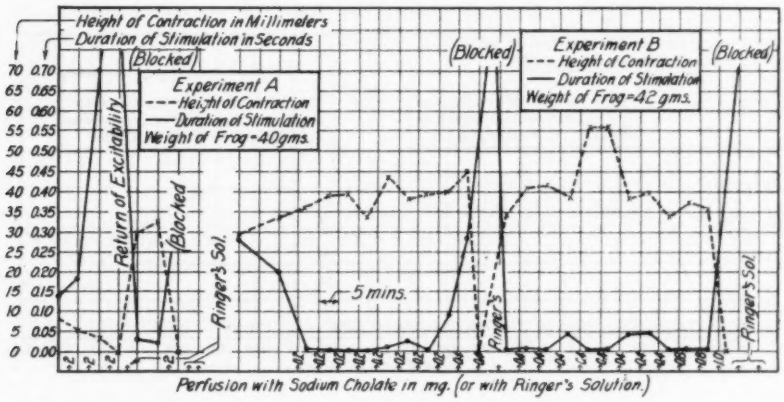

Fig. 4. Perfusion of a reflex gastrocnemius preparation with Na-cholate. The vessels of the leg were ligated.

decrease in the height of the reflex contraction with an increase in the time of stimulation required for response; the depression progressed to complete block. With these larger doses the primary hyperexcitability occurred in 40 per cent of the experiments. In another 40 per cent the depression was immediate and was followed (in one hour) by a variable period of hyperexcitability. It was noted in the latter cases that the heart beat was strong; then there was a second depression followed by block. In 20 per cent of the frogs there was progressive depression from the beginning. The injection of smaller amounts (0.16 mgm. per gram) caused hyperexcitability only (shortened latent period).

A series of experiments was carried out in which the blood supply to the gastrocnemius was not interfered with. Injection of 0.5 mgm. or more per gram of weight gave a picture characterized by depression of the reflex

contraction (fig. 5 B). In some experiments there was a primary period of hyperexcitability of short duration (fig. 5 A). The results obtained from the smaller doses varied. In 2 of 5 experiments there was a primary increase in irritability and for the first 45 minutes after injection the muscle on (reflex) stimulation showed contracture, remaining in this state for several minutes and suddenly relaxing. The duration of the contracture periods gradually decreased. In 2 other experiments the muscle contraction was greater than normal but the duration of the stimulus was longer. In the remaining experiment periods of hyperirritability alternated with periods of depression.

4. THE EFFECT OF BILE SALTS ON THE SKIN RECEPTORS. The reflex time was determined by Türck's method and by electrical stimulation. The foot was then placed in 40 mgm. per cent sodium cholate solution for 30

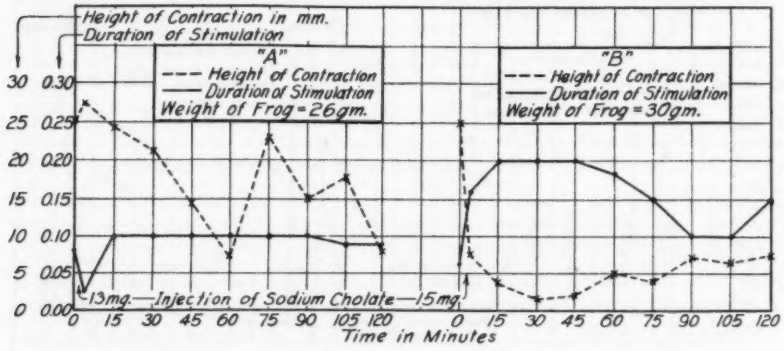

Fig. 5. The effects of injection of Na-cholate on reflex gastrocnemius contraction.

minutes; this caused a prolongation in the reflex time. That some of the salt was absorbed is shown by the fact that the time was prolonged on both sides, but more so in the exposed leg. In experiments in which the vessels of the leg were ligated, the leg exposed to the bile salt solution showed a longer latent period than the non-exposed control leg.

SUMMARY. 1. Injection of bile salts into normal and decerebrate frogs caused a period of motor hyperexcitability followed by depression and diminution or loss of reflex action. The effect depended upon the amount of bile salt injected, the smaller amounts having a tendency to produce hyperirritability, the larger amounts depression.

2. Injection of a small dose of bile salt (up to 0.12 mgm. sod. cholate per gram) with the circulation intact increased the irritability of the muscle-nerve preparation; with the X-injection of larger doses of bile salts depression of the irritability of the muscle-nerve preparation predominated, the

amount of current required for maximal contraction being affected to a greater degree than the threshold. However, the preparation may show a period of hyperexcitability during some phase. With perfusion the results were more constant. Small amounts of bile salts caused hyperexcitability and spontaneous muscle twitches. Larger doses caused depression, contracture and block; but at some stage there may be hyperexcitability even with the larger doses. Curarized muscle showed contracture but no twitching; the effect upon the muscle was but slight. Direct application of bile salt solution to the nerve fibre had no effect unless the salt was in very high concentration, 1.0 per cent.

3. Small doses caused a decrease in the latent period of the crossed gastrocnemius reflex; this could be removed by perfusion with Ringer's. Large doses caused an initial decrease in the reflex time (latent period) followed by a block; in some cases this could be removed by perfusion with Ringer's.

4. Direct application of bile salts (0.04 per cent) to the skin decreased the irritability of the receptors.

CONCLUSIONS

These experiments seem to warrant the following conclusions as to the effect and site of action of purified bile salts on the neuro-muscular system:

1. Bile salts are only slightly toxic to muscle.
2. Bile salts do not materially effect conduction through the nerve fiber.
3. The neuro-muscular junction and the reflex centers of the cord are the structures most susceptible to the toxic effects of bile salts.

The authors wish to express their appreciation to Dr. A. J. Carlson for reading and criticizing this paper and to Dr. R. W. Gerard for helpful suggestions, and for conducting the experiment on oxygen consumption.

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CHANGES IN THE REACTION OF URINE ON AWAKENING

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That the reaction of urine undergoes certain more or less regular changes during the course of the day has been known for about 70 years. As the general history of the study of these changes has been discussed by many recent writers (1), (2), (3), (4) it will not be dwelt upon here. It is sufficient to state that most of the earlier writers attributed the phenomenon to the secretion of acid by the gastric glands. This, they claimed, produced a relative alkalinity in the body which in turn caused the excretion of an alkaline urine. Much recent work, particularly that upon patients whose stomachs do not secrete hydrochloric acid (4), (5), (6) has tended to bear out this view. On the other hand Leathes (7), (8) in 1919, challenged this theory, and suggested that these changes in reaction were due to respiratory phenomena. When a patient awoke from sleep a period of overventilation ensued which caused the excretion of an alkaline urine. His position has recently been supported by other observers (9), (10), and is in accord with the work of various physiologists who have studied related problems. Collip and Backus (11) and Grant and Goldman (12), for example, investigated the effect of short periods of rapid breathing, and found that the excretion of an alkaline urine was one of the results in their experiments, and Simpson (13) showed that there was an accumulation of carbon dioxide in the body during sleep.

It seems therefore that there may be two factors which more or less regularly influence the reaction of the urine, and it is desirable to study them separately. One method of doing this is to study changes induced by respiration in cases where there is no secretion of hydrochloric acid by the stomach, as suggested in an earlier paper (5). It was shown in that publication that the average values obtained upon patients with achlorhydria showed an increasing acidity during the hours immediately after they awoke. Another method would be to compare the reaction of the night urine with that collected before any meal is fed or water drunk, since Bergheim, Rehfuss and Hawk (14) have shown that water alone stimulates gastric secretion. It is data of this latter sort which are presented below.

A series of tests was run upon a few normal subjects and upon many

patients suffering from various gastric disorders. In many of these an absence of hydrochloric acid from the gastric juice was demonstrated by the fractional method (15), (16). Each subject was aroused at 7 o'clock, and the urine in the bladder collected. Although this was doubtless often not the complete night sample, still it should serve satisfactorily for comparison with specimens collected later. Urine was voided again at 8, at 9,

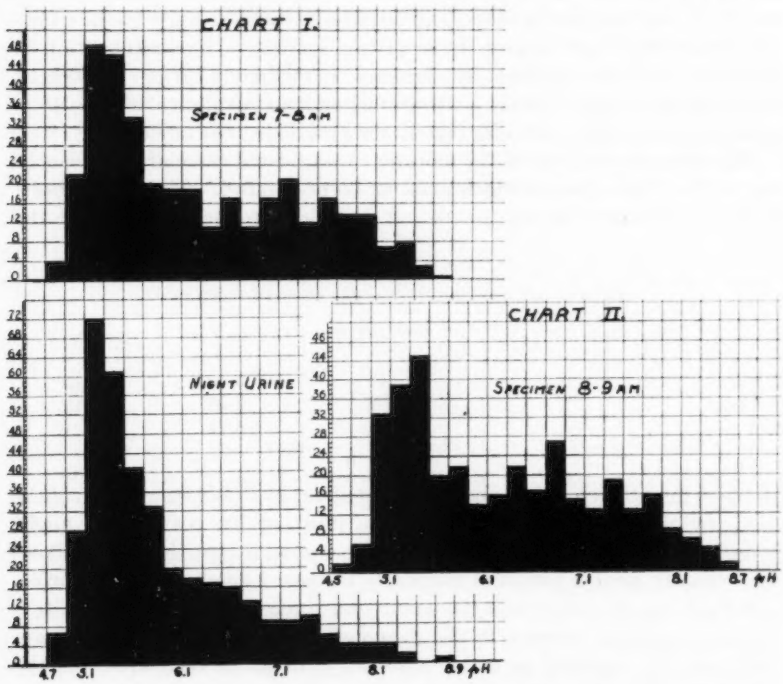

Chart I. Distribution of reactions expressed as pH for night urine specimens and for those collected during the first hour after awakening.

Chart II. Distribution of reaction values obtained during the second hour when a meal was fed.

and at hourly intervals through the morning A meal usually consisting of a glass of milk, a glass of water, two slices of toast with a pat of butter, and an egg was taken between 8 and 9 o'clock. The reaction of the urine was determined colorimetrically after diluting 2 cc. with 8 cc. of distilled water. In the present paper only those subjects from whom a night urine was obtained will be considered, and attention will be centered upon the reaction of that specimen and of those collected during the two following hours.

Distribution curves showing the results are given in charts 1 and 2, and the average and median values in table 1. It is evident that during the hour after awakening, when no food or drink had been taken, the urine was decidedly more alkaline than it was during the night. This change, then, is probably independent of the secretion of hydrochloric acid by the stomach. Since many patients who showed such an increased alkalinity also showed an absence of hydrochloric acid in the stomach it may be said definitely that frequently there is an increased alkalinity early in the morning which does not depend upon gastric activity. The explanation is probably the one offered by Leathes: immediately after awakening accumulated carbon dioxide is blown off, and an alkalinity of the urine results just as it does in similar experiments carried out at any time.

When the second hour of the morning is considered there are, it seems to the author, three factors which must be borne in mind. The first is that a meal was furnished during this period, and that production of acid in the

TABLE 1

Average and median values of the observed reactions

SPECIMEN	OBSERVATIONS NUMBER	AVERAGE pH	MEDIAN pH
Night	371	5.92	5.62
1st hour	367	6.32	6.02
2nd hour	360	6.28	6.10

stomach may therefore have been brought about by direct stimulation of the food, by the formation of the appetite juice, or by both This might cause a reduction in urinary acidity. The second is that this hour follows immediately after a period of markedly increased alkalinity of the urine, and there may therefore be a tendency toward recovery from this condition and a consequent increase in the degree of its acidity. The third possibility can be regarded as a not improbable break in technique, for the subject may have resumed his sleep immediately after waking, and so have begun his activities at 8 o'clock instead of at 7. Such a procedure might delay the adjustment to waking conditions, and produce an additional alkalinity in the 8 o'clock specimen due to respiratory causes. An attempt was made to determine whether this last difficulty had operated to any considerable extent in our series. It was felt that if many patients with an achlorhydria showed an increased alkalinity during the second hour it had arisen from such a delayed adjustment to the activities of the day. When the records were examined it was found that in practically every case where the 9 o'clock specimen was more alkaline than was that obtained at 8 there was hydrochloric acid in the gastric juice. Our studies suggest, therefore, that these changes were due to acid secretion, but it still

seems probable that in some instances an undetected effect of the respiratory cause might have been present.

When the distribution curve of results obtained in the second hour is examined it is evident that it closely resembles that given by the specimens collected between seven and eight. There are, however, a few slight differences between them. There was a decrease in the number of specimens showing the most acid, and the most extreme alkaline reactions. The average and median values (shown in table 1) hint at the same thing. There was a slight increase in the median, and a slight decrease in the average value. These differences are probably not in exces of the allowable limit of error, but they suggest that more subjects showed an alkalinity at this time, but that the degree of the alkalinity was not so marked. If recovery from the respiratory tide had been complicated by the start of another tide due to gastric causes such a relationship would be expected.

TABLE 2

Distribution of differences in reaction between successive specimens of urine

	DIFFERENCES						
	Over +10	+6 to +10	+3 to +5	+2 to −2	−3 to −5	−5 to −10	Over −10
Specimens	No.	No.	No.	No.	No.	No.	No.
Night and 1st	77	59	50	140	21	16	8
1st and 2nd	22	33	30	169	43	38	23

Differences are expressed in terms of 0.1 pH. When an increase in pH was noted it has been given a plus value. Decreases in pH are marked with a minus sign.

The differences in reaction between the night urine and the specimen collected at eight o'clock, and between the eight and nine o'clock specimens was also tabulated. A summary of the results is given in table 2. The marked shift towards an alkaline reaction after awakening is clearly shown. It is also evident that during the second hour there were, besides a fairly large number of cases which remained unchanged or showed a decrease in alkalinity, a large number where the acidity actually increased. As the author is inclined to interpret these results, they show that the alkalinity which developed during the first hour tended to pass off during the second one. The results summarized in table 2 were also plotted upon arithmetical probability paper. These charts showed that the curve of the differences between the first and second hour of the morning was not a true distribution curve, but that various factors were influencing that difference.

Table 2 shows that practically one half of the subjects studied showed an increase in the degree of alkalinity of the urine on awakening, and that over

thirty-six per cent of them showed an increase of over 0.5 pH, a variation which must almost certainly be regarded as significant except, perhaps, when the urine is very alkaline (17). The author has stated his reasons for believing that changes in reaction occurring in the second hour may sometimes also be due to respiratory causes, and has given the explanation of why he does not think that there were many cases in this series where such causes operated. Nevertheless it seems worthwhile to give the per cent of cases which showed changes in either the first or second hour. There were 45 per cent of all tests carried out which showed an increased alkalinity of more than 0.5 pH during one period or the other, while in sixty-one per cent of them there was an increase which was probably greater than the experimental error 0.2 pH. It is not strange that some authors have believed that a change which takes place in such a large number of subjects is the general rule in normal individuals (7), (8), (10) although some of the work upon normal subjects which is available in the literature (18) shows that this is not universally true. It is certain, however, that there is a regular change in the reaction of urine which is almost certainly independent of the secretion of hydrochloric acid by the stomach, and that this change is an important, and in some cases a marked one.

Within an hour after awakening a sigificant increase in the alkalinity of the urine often occurs when no food is eaten and no water drunk. It seems probable that this change is independent of the secretion of hydrochloric acid by the stomach,—a probability which is increased by the presence of the change in patients with achlorhydria. During the second hour of the morning there is a tendency on the part of some subjects toward the excretion of a urine more acid in reaction than that voided immediately after awakening. In the studies reported a meal was fed during this hour, and the gastric stimulation produced seemed to counteract this tendency in many instances.

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DOES ETHYLENE AFFECT RATE OF GROWTH OR ACTION OF ENZYMES IN ANIMALS?[1]

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R. B. Harvey has shown that the presence of a very small quantity of ethylene in the air will cause green fruits and vegetables to ripen in both color and flavor much more rapidly than normally. In fact, if green tomatoes, bananas and other fruits or vegetables are exposed to a concentration of one to one thousand ethylene in air for a period of forty-eight hours at ordinary temperatures, they ripen rapidly, and are in every way indistinguishable from those which have ripened in the ordinary way. This process is so certain in its action that it has attained widespread commercial use; so that these articles of food are shipped into Minneapolis in the green state and ripened artificially in the warehouses.

This action of ethylene in the plant world is so remarkable that we have attempted to discover whether a similar effect is exerted upon the growth of animals, and upon the rate of action of the enzymes of animal tissues.

EFFECT OF ETHYLENE IN THE DRINKING WATER. E. A. Hewitt, in some unpublished experiments done at the University of Minnesota, had allowed a small number of guinea pigs to drink water saturated with ethylene, and thought that he could find some evidence of stimulation of growth; but the guinea pig is extremely lacking in uniformity in growth, and the number of experiments done was too small, to warrant conclusions.

Our experiments were done with rats, as the latter grow much more uniformly. The animals which drank water saturated with ethylene were taken from the same litters, and were always of the same sexes, as the control animals. They were all fed on C. M. Jackson's standard diet, consisting of a mixture of

	grams
Casein	150
Powdered whole milk	100
Butter	52
Sodium chloride	8
Calcium carbonate	15

[1] This investigation was made possible by a grant of funds by the Eli Lilly & Co., Indianapolis, Ind.

This mixture was thoroughly mixed and then mixed with 675 grams whole wheat. Jackson has found this diet adequate in calorie equivalents, vitamines and mineral content.

The drinking water was saturated with ethylene and then placed in flat eight-ounce bottles provided with a doubly perforated cork bored with holes for glass tubing of 6 mm. internal bore. One of these furnished the drinking fountain for the rats. The other tube was connected with an eight-liter bottle filled with pure ethylene which served to keep the drinking water fairly well saturated. The controls received tap water from similarly constructed drinking bottles, but without the ethylene. Both groups of rats drank about 3 ccm. of water apiece per day throughout the experiment.

As the individual growth curves show no striking features during the course of growth, only the figures at the beginning and end of the growth experiment are given, and these are recorded in table 1.

From these figures it seems that ethylene in the drinking water usually exerts a retarding effect on the growth of the rats tested. This is accompanied by a definite lessening in the food consumption. It may, however, not be due solely to the ethylene, but may be due to the un-

TABLE 1

DRINKING WATER SATURATED WITH ETHYLENE (SERIES I)					CONTROLS				
Rat	Initial weight 7/23	Weight 9/9	Gain	Per cent gain	Rat	Initial weight 7/23	Weight 9/9	Gain	Per cent gain
	grams	*grams*	*grams*			*grams*	*grams*	*grams*	
A ♂	44	204	160	363.6	a ♂	48	192	144	300
C ♂	42	220	178	423.8	c ♂	45	204	159	353.3
D ♀	41	163	122	297.5	d ♀	36	154	118	327.7
E ♀	45	172	127	282.2	e ♀	42	162	120	285.7
F ♂	42	178	136	323.8	f ♂	42	198	156	371.4
Average gain.....			144.6	338.2				139.4	327.6

DRINKING WATER SATURATED WITH ETHYLENE (SERIES II)					CONTROLS				
Rat	Initial weight 7/22	Weight 8/30	Gain	Per cent gain	Rat	Initial weight 7/22	Weight 8/30	Gain	Per cent gain
	grams	*grams*	*grams*			*grams*	*grams*	*grams*	
A ♂	41	128	87	212.2	a ♂	44	170	126	286.3
B ♀	40	126	86	215	b ♀	44	166	122	277.3
G ♂	42	126	84	200					
H ♂	44	160	124	281.8	h ♂	37	130	93	256.8
Average gain.....			95.2	227.2				113.7	273.5

TABLE 1—*Concluded*

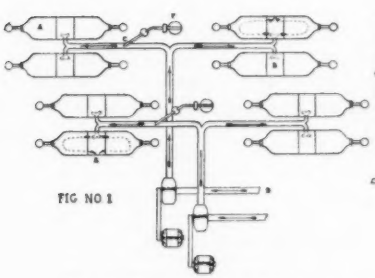
DRINKING WATER SATURATED WITH ETHYLENE (SERIES III) (WISTAR INSTITUTE ALBINO RATS)					CONTROLS				
Rat	Initial weight 8/16	Weight 9/3	Gain	Per cent gain	Rat	Initial weight 8/16	Weight 9/3	Gain	Per cent gain
	grams	*grams*	*grams*			*grams*	*grams*	*grams*	
A ♂	93	124	31	34.2	a ♂	95	160	65	68.4
B ♂	67	91	24	35.8	b ♂	68	124	56	82.3
C ♀	75	92	17	22.6	c ♀	73	104	31	42.4
D ♀	77	97	20	26.0	d ♀	77	128	51	66.2
E ♀	70	108	38	54.3	e ♀	72	120	48	66.7
Average gain.....			26.0	34.6				50.2	65.2

appetizing odor that is maintained in ethylene gas for anesthesia in order to prevent danger and explosions from undetected leaks. In one series of five rats there was a very slight increased growth in the ethylene animals, but this was well within the limits of individual variation, and the loss of weight in the ethylene animals was ten times as great in other repetitions of the same experiment. It is therefore evident that ethylene in the drinking water does not stimulate growth.

EFFECT OF ETHYLENE IN THE INSPIRED AIR. *Method.* The next question was to determine the effect of ethylene in the inspired air, so as to make conditions correspond to those in Harvey's plant ripening process. Technically, this is a much more difficult problem since it is impossible to keep the air in a room saturated with a definite strength of ethylene. Accordingly, the rats were kept in 14 liter chambers made from two truncated 8 liter bottles which were connected end to end by a sleeve of tin.[2] The air entered the chamber through a pipe that penetrated the sleeve, and it left the chamber through an equal sized hole on the opposite side. It was blown at a constant rate by a Sturtevant fan blower through a main air pipe, which then divided by gradually narrowing segments into two; one to the controls and the other to receive the ethylene. In this way the two sets of animals received equal ventilation. The ethylene was released from a small cylinder governed by a Hoke micro-reducing valve (valvo-gauge) and bubbled very slowly through a wash-bottle. The rate of ethylene flow was noted by counting the number of the bubbles, whose size had been calibrated. The ethylene enters the branch of the main air feed tube through a small tube soldered into the side.

[2] The problem of equal ventilation is a difficult one and this arrangement of tubes was designed for us and the calculation of air velocity was made by Mr. Frank W. Morris, late Instructor in Engineering at the University of Minnesota. The later determinations were made by Mr. Ernest G. Alden. To both of these we are deeply indebted for their untiring interest in the solution of our problem.

METHOD OF DETERMINING VELOCITY OF AIR FLOW. A Wahlen gauge (described in University of Illinois Bulletin no. 120) was used to determine the velocity pressure of the air flow in the supply pipe to the chambers containing the animals. To obtain the static pressure a 3/32 inch copper tube was soldered to the air pipe and then the wall of the pipe was drilled through the copper tube which made a smooth surface and prevented errors due to a projecting tube inside of the pipe. The static pressures were taken at two points at right angles to each other in the same plane and perpendicular to the direction of flow. The dynamic pressure was taken at a point in the same plane as the static pressure, and the gauge was connected to give the velocity pressure of the air direct. The cross sectional area of the pipe was divided into four equal concentric areas. A reading was taken



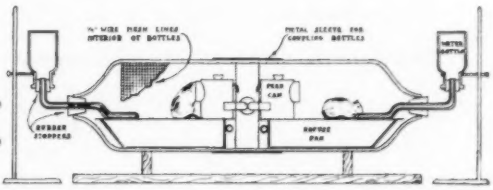


Fig. 1. Diagram showing arrangement of tubes and chambers for testing effects of ethylene in inhaled air.

A, one of the chambers for rat receiving ethylene introduced from cylinder *r* or through inflow tube at *c*.

B, one of the chambers for control rats.

Fig. 2. Sagittal section of a single rat chamber.

at the center of each area on the horizontal diameter, and on each side of the center position, thus giving a total of eight readings from which the mean velocity pressure was obtained. The gauge read direct in inches of alcohol and by means of a precision micrometer, an accuracy of 1/1000 inches of alcohol could be read directly and could be estimated to 1/10,000 inches of alcohol. By means of the following formula the air flow was computed from the readings taken with the Wahlen gauge.

$$Q = AV$$

Where Q = cubic feet of air per second
A = area in square feet
V = velocity in feet per second

$$V = \sqrt{2gh}$$

Where V = velocity in feet per second
g = 32.2 feet per second acceleration of a falling body under attraction of gravity alone
h = head in feet of air at density of air flowing in pipe

To convert inches of alcohol to head in feet of air, the following additional factors must be used.

$$hd = \frac{h_a S k}{12}$$

d = weight of 1 cubic foot air at temperature and pressure in pipe
h_a = head in inches of alcohol
S = specific gravity of alcohol (water = 1.0 at 60°F.) at temperature of gauge
k = weight of cubic feet of water at 60°F.
12 = inches in one foot

Therefore the actual equation becomes,

$$V = \sqrt{\frac{2gh_a Sk}{12d}}$$

and the

$$\text{mean velocity} = \sqrt{\frac{2gSk}{12d}} \times \sqrt{h_a^1} + \sqrt{h_a^2} + \sqrt{h_a^3} \ldots \text{etc.}$$

Where n = number of readings taken for the velocity pressure.

In this way dilutions of ethylene in air, one per cent, one-tenth per cent and one-hundredth per cent, were readily obtained; and the effects of inhaling these concentrations were studied on several series of rats. Typical results may be seen in tables 2 to 5.

The variations found are well within the limit of normal variations. It cannot be said from our experiments that ethylene in any of these concentrations exerts any appreciable effect upon the growth of the rats. This is in striking contrast to the definite effects of ethylene on the ripening of plants.

EFFECT OF ETHYLENE ON THE ACTION OF ENZYMES. Since the ripening of fruit (ester formation) is dependent largely upon the action of enzymes, the effect of ethylene upon the action of the enzymes of animal tissues was investigated. Contrary to our expectations, we were unable to find any effect upon the action of lipase or esterase; nor could we detect any upon pepsin or trypsin; but on the other hand, our experiments indicate that ethylene does cause a definite increase in the action of amylase.

The enzyme experiments were carried out at 37° in a water bath, which was maintained very constantly at this temperature by immersing in it

an electric curling iron. The temperature of the water could be regulated quite accurately by altering the depth to which the curling iron was immersed.

TABLE 2

ONE PER CENT ETHYLENE INHALED					CONTROLS				
Rat	Initial weight 3/15	Weight 4/3	Gain	Per cent gain	Rat	Initial weight 3/15	Weight 4/3	Gain	Per cent gain
	grams	*grams*	*grams*			*grams*	*grams*	*grams*	
A ♂	84	103	19	22.5	a ♂	84	106	22	26.2
B ♀	60	104	44	73.3	b ♀	63	118	55	87.0
					c	68	80	12	17.6
D ♂	66	135	69	102.4	d ♂	50	106	56	112.0
E ♂	66	114	48	72.7	e ♂	75	139	64	85.2
F ♂	59	108	49	83.2	f ♂	65	145	80	123.0
G ♂	63	92	29	46.2	g ♂	65	145	80	123.0
H ♀	34	76	42	122.0	h ♀	75	120	45	60.0
I ♀	60	85	25	41.7	i ♀	58	77	19	32.7
J ♂	40	73	33	82.5	j ♂	37	87	50	134.5
K ♂	35	Died							
L ♀	67	105	38	56.6	l ♀	56	77	21	37.5
Average gain.....			39.6	70.3				45.5	76.2

TABLE 3

1/1000 ETHYLENE INHALED					CONTROLS				
Rat	Initial weight 12/6/28	Weight 2/7/29	Gain	Per cent gain	Rat	Initial weight 12/6/28	Weight 2/7/29	Gain	Per cent gain
	grams	*grams*	*grams*			*grams*	*grams*	*grams*	
A ♂	126	272	146	116.0	a ♂	126	251	125	99.2
B ♀	96	186	90	93.6	b ♀	90	156	66	73.3
C ♀	86	180	94	109.3	c ♀	86	174	88	102.3
D ♀	86	182	96	111.6	d ♀	84	158	74	88.1
E ♂	104	230	126	121.1	e ♂	106	212	106	100.0
F ♀	100	176	76	76.0	f ♀	98	184	86	87.8
G ♀	105	182	77	73.3	g ♀	100	194	94	94.0
H ♂	86	250	164	190.7	h ♂	88	240	152	172.7
I ♀	78	178	100	128.2	i ♀	75	168	93	124.0
J ♂	94	232	138	146.8	j ♂	98	240	142	144.9
K ♂	108	230	122	112.9	k ♂	109	264	155	142.2
L ♂	100	236	136	136.0	l ♂	104	260	156	150.0
Average gain.....			113.7	117.9				111.4	114.9

Ethylene was bubbled through the suspension of enzyme and substrate at a fairly constant rate (a continuous sequence of separate bubbles)

while in order to obtain the same degree of agitation, compressed air was bubbled at about the same rate through the control. That this was very important was shown in one of our earlier experiments in which the ethylene seemed to increase the activity of liver lipase by 50 to 100 per cent; while in later experiments in which the control was agitated, no such effect could

TABLE 4

1/10,000 ETHYLENE INHALED (SERIES I)					CONTROLS				
Rat	Initial weight 2/4/28	Weight 3/20/28	Gain	Per cent gain	Rat	Initial weight 2/4/28	Weight 3/20/28	Gain	Per cent gain
	grams	*grams*	*grams*			*grams*	*grams*	*grams*	
A ♂	46	214	168	365.2	a ♂	44	148	104	236.3
B ♂	44	204	160	363.4	b ♂	43	192	152	353.5
C ♂	33	200	167	506.0	c ♂	32	178	146	459.0
D ♀	40	150	110	275.0	d ♀	41	158	117	283.0
E ♀	52	154	102	196.1	e ♀	50	150	100	200
Average gain.....			141.4	341				123.8	306.3

TABLE 5

1/10,000 ETHYLENE INHALED (SERIES II)					CONTROLS				
Rat	Initial weight 4/6/28	Weight 6/16/28	Gain	Per cent gain	Rat	Initial weight 4/6/28	Weight 6/16/28	Gain	Per cent gain
	grams	*grams*	*grams*			*grams*	*grams*	*grams*	
A ♂	40	236	196	490	a ♂	40	230	190	475
B ♀	25	156	131	524	b ♀	20	170	150	750
C ♀	21	152	131	623.8	c ♀	23	168	145	630.4
D ♂	27	177	150	555.5	d ♂	22	216	194	881.8
E ♀	34	152	118	347.0	e ♀	35	150	115	328.6
F ♂	33	172	139	421.2	f ♂	35	186	151	531.4
G ♂	31	184	153	493.5	g ♂	32	212	180	560.6
H ♀	32	170	138	431.2	h ♀	36	164	128	355.5
I ♂	31	186	155	500.0	i ♂	33	201	168	509.1
J ♀	36	163	127	352.8	j ♀	35	150	115	328.6
Average gain.....			143.8	473.9				153.6	535.1

be noted; and on the other hand, more saturation with ethylene, but without agitation, gave the same low amount of hydrolysis as the unagitated control.

Does ethylene alone tend to hydrolyze starch? In 1927 Helen Rea and R. D. Mullinix published the astonishing statement that ethylene alone caused the hydrolysis of starch which had been exposed to it for periods ranging from 24 to 168 hours at temperatures ranging from 21 to 24°.

However, they do not mention having taken any precautions to prevent the action of bacteria, nor did they make any cultures to indicate any possible rôle played by bacterial growth or agitation.

We have repeated this experiment using one per cent suspensions of starch (U. S. P. amylum, cornstarch) in water in cotton stoppered flasks. These solutions were autoclaved before the ethylene was bubbled through them for twenty-four hours. They were then tested for sugar by the Folin-Wu method, after making sure that neither the ethylene nor the starch affected the Folin-Wu reagents. In no case did either the ethylene treated tubes nor the controls reveal the presence of sugar. The experiment was repeated four times.[3]

We must therefore conclude that some error, perhaps due to bacterial action, has entered into the experiments of Rea and Mullinix, and that ethylene alone does not bring about the hydrolysis of starch.

Amylase. Pancreatic amylase, on the other hand, seems to be definitely activated by ethylene.

1. One gram of starch + 0.1 gram Eli Lilly & Co. powdered pancreatin in 50 cc. H_2O + 5 drops toluene, were placed in one flask through which ethylene was bubbled; and equal quantities of these substances were placed in the control flask through which air was bubbled at approximately the same rate. Both flasks were placed in a water bath at 40°C. for 24 hours.

After 24 hours the contents of the flasks were filtered through a Gooch crucible, washed, dried at 100° to constant weight, and weighed.

Residue (undigested starch)
Ethylene flask = 0.2765 gram
Control = 0.4318 gram

In this it is evident that considerably more starch was converted into dextrins and sugar in the ethylene flask than in the control. The dried portion of the former was also considerably more orange colored than the latter.

2. Into each of two flasks were put the following substances: 1 gram starch, 100 mgm. Eli Lilly & Co. powdered pancreatin, 50 cc. H_2O, 5 drops toluene. Both were kept in H_2O bath at 40° for 24 hours, but through one, ethylene was bubbled, and through the other, air was bubbled at the same rate. At the end of 24 hours the samples were removed, filtered and washed, and the filtrates diluted to 4000 cc. Sugar in the diluted filtrate was then determined by the Folin-Wu method:

[3] We are informed by Dr. L. O. Regeimbal of the Division of Plant Pathology, University of Minnesota, that he also has repeated the experiments of Rea and Mullinix with equally negative results.

1. Sugar in ethylene tube—0.468 gram
   Control 0.292 gram
   Activation by ethylene = 62 per cent

This experiment was repeated twice:

2. Ethylene tube—0.42 gram
   Control 0.32 gram
   Activation by ethylene = 33 per cent
3. Ethylene tube—0.496 gram
   Control 0.346 gram
   Activation by ethylene = 43 per cent

These results would indicate that pancreatin, in contrast to the other enzymes which were tested, is definitely activated by saturation with ethylene.

Pepsin. Into each of two test-tubes were placed 1 gram powdered beef fibrin, 5 cc. 0.3 per cent HCl + 100 mgm. Eli Lilly & Co. pepsin, with 5 drops of toluene added. Both were placed in a water bath at 40°, air bubbled through the control for one hour, and ethylene through the other tube. After one hour's exposure, the contents were filtered in a Gooch crucible, washed and dried at 100° to constant weight.

Weight of undigested fibrin:

	Ethylene tube	Control
1.	0.2428 gram	0.2154 gram
2.	0.2397 gram	0.2190 gram

In experiment 3 and 4 the digestion mixture was diluted up to 25 cc. with 0.3 per cent HCl, and the digestion continued two hours. Conditions were otherwise the same.

Weight of undigested fibrin:

	Ethylene tube	Control
3.	0.1586 gram	0.1627 gram
4.	0.1817 gram	0.1966 gram

Trypsin. Into each tube were placed: 2 cc. 4 per cent casein in 0.5 per cent Na_2CO_3, 3 cc. 10 per cent Eli Lilly & Co. pancreatin, 5 drops of toluene. Ethylene was bubbled through one tube, air through the other. Both tubes were kept at 40°C.

After 48 hours, the contents were diluted ten times and an 8 cc. sample analyzed for nitrogen by the method of Van Slyke:

Control tube—12.7 cc.
Ethylene tube—13.5 cc.

In another experiment we took: 150 cc. 4 per cent casein in 0.5 per cent Na_2CO_3 and 1.5 cc. powdered trypsin (Fairchild & Foster), 5 drops toluene.

One tube was saturated with ethylene and kept in an atmosphere saturated with ethylene, but the ethylene was not bubbled through, while the control was kept in ordinary air.

Nitrogen (determined by Van Slyke method) in a 4 cc. sample:

	Ethylene tube	Control
After one hour	13.6 cc.	13.0 cc.
After three hours	16.2 cc.	15.7 cc.
After seven hours	18.3 cc.	17.0 cc.
After nineteen hours	22.8 cc.	21.8 cc.
After twenty-four hours	23.7 cc.	23.6 cc.

Lipase. The effect of ethylene on lipase was determined by the method of Kastle and Loevenhart, using the hydrolysis of ethyl butyrate by the lipase made from the liver of dogs which had been killed suddenly by a blow on the head without etherization. The hashed liver was extracted with equal parts of glycerine and water to make up a 40 per cent extract.

1. Into each tube were put: 10 cc. 40 per cent liver extract 2.6 cc. ethylbutyrate, 40 cc. H_2O, and 5 drops toluene. Ethylene was bubbled through one, air at the same rate through another. Both tubes were kept one hour at 40°. Equal samples were then titrated with N/20 NaOH, using phenolsulphonephthalein as indicator and titrating the brown suspension to a faint pink.

Ethylene tube acidity equivalent to 104.3 cc. N/20 NaOH
Control tube acidity equivalent to 107.3 cc. N/20 NaOH
Previously
boiled control acidity equivalent to 8.6 cc. N/20 NaOH

2. Five cubic centimeters 40 per cent liver extract, 2 cc. ethylbutyrate, 40 cc. H_2O, 1 drop toluene; ethylene and air bubbled as before. Kept at 40° for one hour.

Ethylene tube acidity equivalent to 58.3 cc. N/20 NaOH
Control tube acidity equivalent to 57.7 cc. N/20 NaOH
Boiled
control tube acidity equivalent to 6.0 cc. N/20 NaOH

3. Four samples were taken, each containing 1 cc. 20 per cent dog's liver suspension, 1 cc. ethylbutyrate, 40 cc. H_2O, 1 drop toluene. Through one, ethylene was bubbled, and through its control, air was bubbled at the same rate. The other tubes were not agitated by bubbling, but one was saturated with ethylene while the other was not. All four were kept at 40° for one hour. The results were as follows:

Tubes agitated by bubbling—
Ethylene tube equivalent to 54.7 cc. N/20 NaOH
Control tube equivalent to 52.7 cc. N/20 NaOH

Non-agitated controls—
Ethylene tube equivalent to 30.7 cc. N/20 NaOH
Control tube equivalent to 31.2 cc. N/20 NaOH

The effect of agitation, where better mixing of the ethylbutyrate is obtained, is evident; but the ethylene itself has no effect.

4. As might be expected, when the ethylbutyrate is exposed for a longer time, the effect of agitation is not noticeable. Thus, in another experiment which lasted 20 hours:

Tubes agitated by bubbling—
Ethylene tube equivalent to 35.8 cc. N/20 NaOH
Control tube equivalent to 34.9 cc. N/20 NaOH

Non-agitated controls—
Ethylene saturated equivalent to 34.4 cc. N/20 NaOH
Control 38.1 cc. N/20 NaOH

Similarly negative results were obtained with lipase when cottonseed oil emulsified with acacia (Morrow) was used instead of ethylbutyrate.

Ten cubic centimeters cottonseed oil were emulsified with 7.5 grams acacia in 250 cc. H_2O, 2 cc. 6 per cent steapsin (Digestive Ferments Co.) were added, and 20 cc. 0.25 per cent formalin added to kill the ferments. Twenty-five cubic centimeter samples were used. They were kept at 40°C., and before titrating 50 cc. of alcohol were added to dissolve the fats. Ethylene was bubbled through one set of tubes, and air through another.

After 20 hours,
Ethylene tube equivalent to 6.5 cc. N/10 KOH
Control tube equivalent to 6.5 cc. N/10 KOH

After 24 hours,
Ethylene tube equivalent to 7.1 cc. N/10 NaOH
Control tube equivalent to 7.0 cc. N/10 NaOH

With liver lipase the emulsified cottonseed oil gave similar effects.

Emulsified cottonseed oil 50 cc., 10 cc. 40 per cent suspension of dog's liver, 2 cc. ethylbutyrate. Ethylene was bubbled through one tube, and air through another; kept 2 hours at 40°C.

Ethylene tube equivalent to 38.0 cc. N/20 NaOH
Control tube equivalent to 37.3 cc. N/20 NaOH

We also made several attempts to determine whether saturation with ethylene would enhance the synthesis of ethylbutyrate by liver lipase from alcohol and butyric acid, as had been shown for normal liver extract by Loevenhart with ethylbutyrate, and by Hanriot with monobutyrin. We found that it was not possible to do this successfully on a quantitative

basis owing to the presence of too many other fats and protein in the liver extract, and also to the ease with which the ethylbutyrate is hydrolyzed by mixtures of very slight acidity or alkalinity, especially at the temperature of steam distillation. We could, however, find no difference in the odor of the two samples, indicating that very little synthesis had taken place, and no difference in the odor of the first parts of the steam distillates. There is therefore no definite evidence of a difference in ethylbutyrate synthesis under the influence of ethylene.

SUMMARY

Our experiments show no stimulating effect on the growth of rats by either drinking water saturated with ethylene or by inhaling over long periods air containing one per cent, one-tenth per cent, or one-hundredth per cent of ethylene. The growth of rats is therefore not favorably affected by ethylene in ten times the concentration which activates ripening of plants.

Saturation with ethylene does not convert starch into sugar as claimed by Rea and Mullinix. It does, however, increase the activity of amylase, but does not affect the activity of pepsin, trypsin or liver lipase. We were not able to note any marked effect in the reversal of lipase action, i.e., the synthesis of ethylbutyrate from ethyl alcohol and butyric acid.

It is a pleasure to express to Drs. R. B. Harvey, L. O. Regeimbal, C. M. Jackson, F. Morris and E. Alden our thanks for their kind suggestions and advice, to Mr. Grant Christianson for assistance in some of the experiments, and to the Eli Lilly & Co. for their generosity in the granting of funds with which to carry on the work.

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STUDIES ON THE FETAL CIRCULATION OF MAMMALS[1]

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The work of Pohlman and the more recent investigations in this laboratory have shown that new methods must be applied to studies of the fetal circulation before our knowledge will be adequate. In a recent publication (Kellogg, 1928) we dealt with the course of the blood flow through the fetal mammalian heart and its degree of mixing there. The present investigation not only extends that work but in addition will consider the gaseous exchange in the placenta and the gaseous content of the blood carried within the fetal vessels.

The history and development of the theories pertaining to the fetal circulation have been extensively reviewed by Pohlman (1909) and the more important phases have also been discussed in the previous contribution by the present writer (Kellogg, 1928). For these reasons it will be sufficient to make merely a brief statement of the problem as it now stands.

Sabatier (1791) elaborated his conception of the course of the two caval streams through the heart. On purely anatomical grounds he described the inferior caval stream as being shunted by the Eustachian valve through the foramen ovale and so into the left atrium; similarly, he postulated the superior caval stream as being directed to the tricuspid orifice by the intervenous tubercle. Thus, two relatively slow moving blood streams were supposed to cross within the right atrium without mixing. It is evident that such an arrangement would give the head and upper extremities a purer blood supply than that received by the rest of the body. Since its appearance, this theory has gained general favor; using it as an hypothesis, the more rapid and earlier development of the liver, head and upper extremities has been explained as due to their presumptive superior oxygen supply.

Pohlman (1907; 1909 a, b) took exception to the Sabatier conception and was the first to present evidence based on experimentation with living animals. By injecting a starch suspension in one of the caval streams, followed by its subsequent recovery simultaneously from the two ventricles,

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he demonstrated that the two caval streams do mix in the right atrium. While Pohlman's experiments were original, carefully performed and up to that time the only work done with living fetuses, it has not met with much acceptance. Perhaps this is due to the fact that the number of animals used was small.

Since Pohlman's results were contradictory to the accepted Sabatier theory of the course of the caval stream within the heart, and also because they have an important bearing on the more fundamental problem of the courses of the differential ratio of growth of the various parts of the fetus, it seemed important to repeat and if possible extend them. To this end a large number of living pig fetuses and a small number of living dog fetuses were injected using the Pohlman technique except for some slight modifications. The results of this work are published in the communication already mentioned and it is sufficient to state here that they confirmed and strengthened the conclusions of Pohlman in every detail.

Although convinced that the starch injections gave results closely representing the actual conditions in the fetal heart, it was realized that there are certain theoretically objectionable features to that type of experimentation. Since it was also desirable to secure a more strictly quantitative method, resort was made to a chemical analysis of the gases carried by the blood. The Van Slyke (1924) micro-method of gas analysis proved ideal for this type of work, and in addition to giving information regarding the course of the two caval streams within the heart it also yielded some information relative to the question of fetal respiration and the gaseous exchange in the placenta.

MATERIAL AND METHODS. Living pregnant dogs were used in these experiments because the value of the gas analysis is dependent upon the maintenance of a normal placental relationship between the mother and the fetus. Under conditions which were as closely normal as possible samples of blood were taken from the uterine artery of the mother and from the great vessels of the fetus. The oxygen and carbon dioxide content of these samples were determined by the micro-adaption of the Van Slyke method (1924).

Since volatile anesthetics markedly affect the results of gas analysis of the blood, only local anesthetics were used. The first eight dogs were operated under an anesthetic consisting of one-fourth grain of morphine and one-hundredth grain of scopolamine, given one half hour before the operation, and one-two hundredth grain of scopolamine at the start of the operation. In addition, the line of incisions was infiltrated with a one-half per cent solution of procaine. The last two dogs used were given a spinal anesthetic according to the following technique: twenty to forty cubic centimeters of a three-fourths per cent procaine solution were injected intra-durally at the level of the third or fourth lumbar vertebra

and the head of the animal held in an elevated position to prevent bulbar paralysis which might have occurred if the injection had reached the medulla. The line of incision in the abdomen was also infiltrated with a one-half per cent procaine solution. Both types of anesthetic were found to be efficient and caused no noticeable effects upon the respiration of the mother or the heart rate of either mother or fetus. Local anesthetics are especially good in this type of experimentation because they do not affect the blood pressure of the animal and there is no indication that they alter the results of gas analysis.

The dog's abdomen was opened with a long incision in the mid-line and one fetus at a time brought to the surface. To reduce hemorrhage to a minimum the uterus was always opened between the adjoining placentas since this region is the least vascular. As the fetus was taken from the amniotic sac the portion of the membrane surrounding the head and neck was held tightly in place and allowed to remain about the head; this served effectually to prevent the filling of the lungs in case an attempt to breathe was made. The skin was cut the full length of the sternum and the pectoral muscles were then torn away by blunt dissection. One point of a sharp hemostat was next thrust beneath the sternum and internal mammary arteries and clamped tightly to shut off these vessels. It was then possible to cut away the sternum and such parts of the ribs as were necessary to expose freely the heart; with this procedure only slight hemorrhage resulted. Care was taken to see that the umbilical cord was not occluded by twisting or tension before any samples of blood were taken. All samples were drawn under oil and transferred to small tubes containing crystals of oxalate under oil, so that at no time was the blood exposed to the air.

The first few samples taken from the two ventricles were drawn simultaneously; this proved to be difficult because the vessels readily collapsed if the blood was drawn even moderately fast. Consequently, thereafter a sample was first obtained from one ventricle and then after a short wait, sufficient to allow the circulation to readjust itself, the second sample was drawn from the other ventricle. The first sample was taken alternately from the right or left ventricle to overcome any inequality that might occur as a result of the first sample being drawn continuously from the same side of the heart. The average sample was about three-fourths of a cubic centimeter; this amount is well within the limits of safety since the removal of about three cubic centimeters of blood is necessary to cause collapse of the umbilical vessels. All samples were drawn very slowly to avoid the possibility of sucking blood through the foramen ovale from the opposite ventricle.

The small amount of blood available in the fetal heart made it necessary to use the micro-variation of the Van Slyke method of analysis. This

method requires but two-tenths of a cubic centimeter of blood for a single determination. Duplicate analyses were made on all samples which were sufficient in quantity and the average of the two determinations accepted. The accuracy of the method and the calibration of the apparatus were checked by running controls from non-pregnant dogs. The oxygen and carbon dioxide content of these samples lay well within normal limits.

TABLE 1

Results of oxygen and carbon dioxide analyses of blood taken from the right and left ventricles of dog fetuses

DOG NUMBER	FETUS NUMBER	RIGHT VENTRICLE O_2	LEFT VENTRICLE O_2	RIGHT VENTRICLE CO_2	LEFT VENTRICLE CO_2	WEIGHT
		vol. per cent	*vol. per cent*	*vol. per cent*	*vol. per cent*	*grams*
1	1	1.65	3.35	37.80	36.88	
1	2	1.72	1.69	35.04	34.21	
1	3	1.96	2.41	33.97	31.04	
1	4	4.35	2.16	34.77	33.78	
1	5	2.20	2.11	36.20	37.03	
1	6	1.81	1.63	35.84	36.35	
1	7	1.81	1.72	34.56	34.78	
Average.....		2.21	2.15	35.45	34.89	154
2	1	2.85	3.13	49.33	46.33	
2	2	2.55	2.70	47.84	45.80	
2	3	2.40	2.25	53.40	52.78	
2	4	2.69	3.07	51.83	49.17	
2	5	3.58	3.88	53.86	48.34	
Average.....		2.81	3.00	51.25	48.58	200
3	1	2.55	2.80	51.54	47.78	
3	2	1.80	2.11	49.44	49.76	
3	3	2.13	1.95	47.77	50.65	
3	4	1.96	1.96	54.09	48.45	
Average.....		2.11	2.20	51.21	49.16	240
Grand average.....		2.38	2.43	44.33	42.69	

Additional determinations on a sample as a rule showed but little variation and frequently gave identical results.

All reagents (except the sodium hydroxide used for absorption of the carbon dioxide) were extracted before they were used to remove the gases in physical solution. It was not considered necessary to extract the sodium hydroxide because but two-tenths of a cubic centimeter was introduced into the extraction chamber.

OBSERVATIONS. 1. *Results of gas analysis on blood from the heart.* Table 1 gives the oxygen and carbon dioxide analysis of blood taken from the

two sides of the heart of sixteen fetuses. The average oxygen content of blood from the right ventricle was 2.38 per cent while that of blood from the left ventricle was 2.43 per cent. This indicates that there is about 2.10 per cent more oxygen in the blood of the left ventricle than in that of the right ventricle. The carbon dioxide average for the right ventricle was 44.33 volumes per cent and the left ventricle average was 42.69 volumes per cent. The difference here is 3.80 per cent in favor of the right side.

TABLE 2

Results of gas analyses of blood from the umbilical vein of dog fetuses

DOG NUMBER	FETUS NUMBER	UMBILICAL VEIN O_2	UMBILICAL VEIN CO_2	WEIGHT
		vol. per cent	*vol. per cent*	*grams*
1	5	5.57	31.20	150
1	7	8.12	35.12	160
Average		6.84	33.10	155
2	1	10.48	40.71	215
4	2	5.07	44.49	100
5	2	6.68	45.31	
9	1	2.41	47.65	60
9	2	2.72	45.40	65
9	3	2.05	47.78	60
9	4	5.73	44.43	75
9	5	2.27	45.22	65
9	6	2.42	42.47	55
9	7	2.61	41.66	55
Average		2.86	44.94	61
8	3	8.44	46.43	235
8	4	11.74	46.17	290
Average		10.09	46.30	

The individual fetuses showed variations with the greater amount of oxygen and carbon dioxide sometimes on one side and sometimes on the opposite side of the heart. It is noticeable that the oxygen content of the three groups of fetuses was very similar but that the carbon dioxide content of the first group was about thirty per cent lower than that of the second and third groups. This difference of carbon dioxide content can not be correlated with the difference in the weight of the fetuses and the explanation is not apparent; possibly it was due to a difference in combining power of the blood.

TABLE 3

Results of gas analyses of blood from the superior and inferior venae cavae of dog fetuses

DOG NUMBER	FETUS NUMBER	INFERIOR VENAE CAVAE O_2	SUPERIOR VENAE CAVAE O_2	INFERIOR VENAE CAVAE CO_2	SUPERIOR VENAE CAVAE CO_2	WEIGHT
		vol. per cent	*vol. per cent*	*vol. per cent*	*vol. per cent*	*grams*
4	1	3.03		49.25		
4	3	2.27		54.50		
4	4	2.11		51.75		
4	5	2.30		52.08		
4	6	2.13		42.44		
4	7	2.29		44.16		
4	8	2.15		40.54		
4	9	2.76		46.61		
Average		2.38		47.66		100
5	1		3.03		50.62	
5	4		2.91		52.32	Good size
5	6		3.40		50.64	
Average			3.13		51.19	
6	1		5.37		41.21	
6	2		2.25		48.65	
6	3		2.33		44.85	
Average			3.31		44.90	290
7	1		2.87		45.86	
7	2		2.12		44.57	
7	3		2.42		44.73	
7	4	3.63		43.28		
7	5	3.03		46.99		
7	6		2.12		46.51	
Average		3.33	2.38	45.13	45.41	180
8	1		2.13		51.52	
8	2	2.13		52.39		
8	3		2.44		55.08	
8	4	3.48		46.37		
Average		2.80	2.28	49.43	53.30	253
7 and 8, average		3.02	2.35	47.25	48.38	210
10	1	3.30		49.92		
10	2	3.14		49.29		
Average		3.22		49.60		260

2. *Results of gas analysis of blood from the umbilical vein.* Table 2 gives the values for the oxygen and carbon dioxide content of blood taken from the umbilical veins of sixteen fetuses. In the dog the average oxygen content in this series was 5.45 volumes per cent and that of the carbon dioxide 43.41 volumes per cent. In the guinea pigs the oxygen averaged 9.89 volumes per cent and the carbon dioxide 41.34 volumes per cent. There was a marked variation in the oxygen content in the umbilical vein blood of fetuses of different litters. Group nine (dog) averaged only 2.86 volumes per cent while the other groups averaged much higher. It is worthy of note that the fetuses of group nine averaged 61 grams in weight while the others were much larger. The guinea-pig fetuses were at term and consequently compare favorably with the larger dog fetuses.

3. *Results of gas analysis of blood from the superior and inferior venae cavae.* Table 3 gives the analyses of both superior and inferior venae caval

TABLE 4

Results of gas analyses of blood from the uterine artery and vein of the pregnant dogs used in obtaining data of tables 1–3

DOG NUMBER	UTERINE ARTERY O_2	UTERINE ARTERY CO_2	UTERINE VEIN O_2	UTERINE VEIN CO_2
	vol. per cent	*vol. per cent*	*vol. per cent*	*vol. per cent*
2	12.61	37.00	9.93	39.73
5	14.88	37.93		
8	13.44	31.81		
9	12.76	33.05	8.00	38.64
10	11.36	38.36	10.20	41.61
Average.....	13.01	35.63	9.37	39.99

blood; the results of these two sets are combined in one table for the purpose of simplifying comparison. Considerable variation is noticeable in the oxygen content of the different groups. Group 4, which consisted of small fetuses averaging 150 grams in weight, shows less oxygen in the inferior vena cava than was present in the superior vena cava of groups 5, 6, 7 and 10. The latter were large fetuses. In the case of groups 4, 5 and 6 blood was taken from but one vessel (the same vessel in all fetuses of a group) of each fetus. It was hoped that by using all of the fetuses of one group for samples from the inferior vena cava and all of those of the next group for samples from the superior vena cava that the average of the groups could be considered as representing the conditions in the vessel from which the blood was taken. These group averages could then be compared to ascertain the relative amounts of oxygen and carbon dioxide in the superior and inferior venae cavae.

Since the oxygen content of the superior vena cava of groups 5 and 6

averaged more than that of the inferior vena cava of group 4 it is obvious that such comparisons are useless and that comparisons of the oxygen and carbon dioxide content should be made from results obtained on blood taken from the same fetus or at least from fetuses of the same litter.

The animals in groups 7 and 8 were about the same size, and samples were taken from either the superior or inferior vena cava in about one half of the cases in each group. The oxygen averaged 3.02 volumes per cent in the inferior vena cava and 2.35 volumes per cent in the superior vena cava. The inferior vena cava therefore apparently contained about 28 per cent more oxygen than the superior vena cava. The superior vena cava contained 2.3 per cent more carbon dioxide than the inferior vena cava.

4. *Results of gas analysis of blood from the uterine artery and vein of the mothers.* Table 4 lists the values of the oxygen and carbon dioxide content of the uterine artery blood of five mothers and in one case blood taken from the uterine vein. The uterine artery blood contained 37.8 per cent more oxygen than the uterine vein while the carbon dioxide content of the vein was 12.2 per cent higher than that of the artery.

A comparison of table 4 with table 2 shows that in the very small fetuses the fetal blood leaving the placenta contains a much lower oxygen content than that of the uterine vein, whereas the former increases steadily until the valves are nearly equal in fetuses near term.

Discussion. Gas analysis is undoubtedly the most accurate means of determining the relative amounts of oxygen distributed to the various parts of the body and also is probably the best method of determining whether or not the superior and inferior caval blood mix in the right atrium.

The results in the analysis of blood from the two sides of the heart show that the amount of oxygen in the blood entering the aorta and pulmonary artery is practically the same. This proves beyond a doubt that the two caval streams do mix in the right atrium. However, it does not prove that the mixture is exactly equal, because although the blood that passes into the left atrium is diluted by the pulmonary return, yet in spite of this, the blood which reaches the left ventricle is at least equally if not more highly oxygenated than that of the right ventricle. The ratio of oxygen in the inferior vena cava to that of the superior vena cava (see p. 643) would indicate that the higher oxygen content of the inferior caval blood might well take care of the pulmonary return even though the predominence of inferior vena cava blood passing through the foramen ovale were small.

Since the relationship between the pulmonary return and the rate of flow through the left ventricle is not known, it is impossible to determine just what the dilution of the blood through the foramen ovale is, and it is therefore impossible by this means to know exactly what the relative quantities of superior and inferior vena caval blood are that go through

the foramen. It seems safe to assume that the pulmonary return is but a small part of the blood brought to the left ventricle, and for this reason its rôle in venous dilution is not great; if this be admitted, it follows the mixture of blood passing through the foramen ovale is nearly equal in oxygen content to that of the left ventricle.

While it is impossible, as Pohlman has so well urged, to imagine the two caval streams crossing in the right atrium without mixing, it is not at all surprising to find a slightly higher oxygen content in the blood of the left ventricle. The anatomical arrangement certainly favors such a condition because the foramen ovale is closer to and in a more direct line with the inferior vena cava.

The problem of the placental exchange and the relative amounts of oxygen and carbon dioxide present in the blood of the various vessels of the fetus is a difficult one because, as has already been pointed out, it is impossible to take samples from more than one vessel without danger of upsetting the circulation to such an extent that the results of the gas analyses become unreliable. An attempt to overcome this difficulty was made by using single samples obtained from alternate fetuses of the same litter or of litters in which the fetuses were of about the same weight. It is believed that the comparisons thus obtained are reliable.

The results of the analyses of blood from the umbilical vein, superior and inferior venae cavae are suggestive of the amounts of oxygen and carbon dioxide that are probably present in these vessels. They cannot, however, be considered as extensive or consistent enough to be accepted as showing the exact relationships. The general trend of the results is about what might be expected.

The [illegible] for the oxygen and carbon dioxide content of the uterine artery might at first appear to be very low but it has been shown that during pregnancy the total blood volume is increased as much as forty-six per cent (Salvesen, 1919). This increase in volume is not accompanied by any corresponding change in the oxygen and carbon dioxide carrying capacity. On the contrary, the total oxygen and carbon dioxide carrying capacity remains unchanged so that there is really a relative decrease in the carrying capacity per cubic centimeter of blood.

If 20 volumes per cent be considered as the usual value of oxygen in the arterial blood of a non-pregnant dog, then the average of 13.01 volumes per cent indicates that the blood volume must have increased about thirty-three per cent in this series of dogs. It has been shown that the blood volume output per minute of the heart is increased from 25 to 50 per cent during pregnancy (Standard, Duncan and Sisson, 1926). This increase in heart output serves to compensate for the low oxygen-carrying capacity of the blood.

Table 2 indicates that the older the fetus the higher the oxygen content

of the umbilical blood. For example, the oxygen content of the blood from fetuses weighing about 250 grams is nearly three times as great as that of fetuses weighing 60 grams. The results for guinea-pig and dog fetuses about at term were very similar. The values for the content of umbilical vein blood in the larger fetuses closely approaches those obtained for the uterine vein blood of the mothers. These findings seem to indicate that the placenta is able to take care of a greater demand for oxygen during the latter part of gestation than during the early period. The true explanation is not apparent but there may be several factors whose influence might account for the changes. One speculates as to whether fetal blood acquires an increasingly greater oxygen carrying capacity as gestation advances. The placental area is probably the most important factor in the oxygen exchange. It is well known that as the period of gestation progresses the chorionic villi become larger and more branched. This undoubtedly enlarges the effective area for gaseous exchange. Whether or not the placental area per unit weight of the fetus increases near term has not been determined but it seems likely that this does occur.

The question of the efficiency of the placenta as a means of gaseous exchange is an interesting but difficult one. Analysis of blood from very small fetuses would indicate that the efficiency is very low while those on fetuses at term seem to show that the fetal blood oxygen content is approximately that of the venous blood leaving the uterus. It apparently follows that a greater efficiency in terms of the per minute volume output of the placenta would only be secured by a greater per minute intake to the uterus. It seems evident that the placental exchange is not as rapid as that in the lungs and certainly could not meet great variations in demand as the lungs are capable of doing. The oxygen content of arterial blood in the human newborn, after adjustment to the initial cyanosis due to delivery has been effected, is about twice that of the blood in the umbilical vein of the term fetus. The pulmonary area of the newborn is about $\frac{1}{10}$ to $\frac{1}{20}$ of the adult area or 10 to 20 square meters while that of the placenta is but 6.5 square meters (Dodds, 1922). Probably the whole of the placental area is constantly functioning whereas considerable of the pulmonary area is utilized only at times of increased demands for oxygen.

Still another factor plays an important part in the efficiency of the placenta. The speed with which the blood flows through any capillary bed will have a marked effect on the gaseous exchange taking place. In some unpublished work, Farmer and De Takets have shown that slight impediment to the return of venous blood will result in the surrounding tissues removing much larger quantities of oxygen from the blood than normally. The same factors undoubtedly would operate in the placental exchange but in the opposite direction. That is, rapidly flowing fetal blood would not be exposed sufficiently long to the maternal blood for

equalization of gaseous content to occur. A sluggish fetal circulation by this reasoning should have a higher oxygen content in the umbilical vein than does a rapid one. Just such results were obtained in a number of instances where more than one sample of blood was taken from the same fetus with a resulting drop of blood pressure after the first sample. The second sample in these cases contained a higher oxygen content than the first. Such analytic values were not included in the tables because it was apparent that they did not represent true conditions. It is evident then that there must be some optimum rate of flow through the placenta at which rate the quantity of oxygen delivered to the fetus per minute would be the maximum.

A comparison of the carbon dioxide content found in the samples taken from young and old fetuses would indicate that even in the youngest the carrying capacity is high. The relatively high amount of carbon dioxide as compared to that of oxygen, especially in young fetuses, raises the question as to whether it is a matter of retention in the fetal blood stream or whether it might partly be accounted for by the production of carbon dioxide from oxygen liberated in the process of fat storage from carbohydrates. It is possible that this mechanism might be the source of considerable oxygen for the fetus.

CONCLUSIONS

1. The oxygen content of the blood within the two ventricles of dog fetuses as determined by the Van Slyke method is practically the same. It averages about 2.41 volumes per cent.

2. The two caval streams mix thoroughly in the right atrium.

3. The early rapid development of the liver, head and upper extremities as compared to the growth of the rest of the body does not depend upon a superior oxygen supply.

4. The oxygen content of the umbilical vein blood varies greatly with the size of the fetus. It increases progressively with growth and does not approximate that of the uterine vein until fetuses are near term.

5. In the fetus at term the area of the placental surface and the rate of blood flow through the placenta are so balanced as to give a maximum efficiency at which the blood in the fetal umbilical vein is approximately the same in oxygen tension as the venous blood of the mother's uterus.

6. Apparently the higher oxygen content of the umbilical vein blood in fetuses near term may be largely explained on the basis of an increase in placental area.

7. Although the carbon dioxide content of fetal blood is relatively high at all ages, there is little difference between very young fetuses and those at term.

This investigation has been pursued under the direction of Prof. L. B. Arey to whom acknowledgment is due for advice and guidance.

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STUDIES ON THE PHYSIOLOGY OF SECRETIN

III. Further Studies on the Effects of Secretin on the Blood Sugar

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There is not a unanimity of opinions concerning the effects of secretin on the blood sugar. Some authors have described hyperglycemia after the injection of secretin in rabbits, dogs, and man; others have described hyperglycemia followed by hypoglycemia; while another group have observed only hypoglycemia. Mellanby (1928) described a new method of purification of secretin yielding a product which he says possesses great secretagogue activity and no effects on either blood pressure or blood sugar of rabbits or cats. However, Zunz and La Barre (1929; this paper contains a complete bibliography) using a product prepared in Mellanby's laboratory found on injecting large enough doses in dogs to obtain rapid secretion of the pancreas, that there also was a lowering of the blood pressure and sugar. Still and Shipner (1929) reported that highly purified secretin did not possess hypoglycemic properties in normal or diabetic dogs. They used both barbitalized and unanesthetized animals.

One of us (E. U. S., 1929) described a method of preparing a crude secretin which was a powerful secretagogue but possessed no vaso-dilatin. It consisted of precipitating an acid extract of the fresh duodenum with solid NaCl, subsequent solution of the precipitate formed in acid and reprecipitation by trichloracetic acid. This second precipitate was dried by mixing with absolute alcohol and acetone and the addition of ether.

Series I. In this series of experiments we have studied the effects of this crude secretin on the blood sugar. We used chloralosed dogs (80 mgm. in saline per kilo intravenously) having the necessary cannulae in place for the collection of pancreatic juice, samples of blood or injections. A sample of blood was taken, then 1 to 2 mgm. of crude secretin per kilo was injected by vein. Subsequent samples of blood were collected at 15 or 30 minute intervals for 2 hours. Blood sugar determinations were made on Folin and Wu filtrates by the method of Hagedorn and Jensen. Figure 1 shows the results of four experiments of this kind. In these experiments there seemed to be a relationship between the dosage of crude secretin and the change in blood sugar. In experiment I of this group the effects were minimal. The

dog had respiratory difficulties until artificial respiration was resorted to. It is believed the poor results are due to that complication. It is demonstrated by these experiments that crude secretin may be prepared which is a powerful secretagogue, produces hypoglycemia but is not in the least hypotensive. The mechanism of the hypoglycemia was then studied.

Series II. If crude secretin is extracted several times with acidified 90 per cent alcohol the secretin (fraction A) will pass into the alcohol leaving behind a residue (fraction B). *Fraction A, secretin,* on treating with brucine, pyridine and methyl alcohol extraction as described in the first paper of this series yields a white and very active secretagogue which is not in the least hypotensive nor produces hypoglycemia, even in dosages of 3 to 5 mgm. per kilo. Figure 2 shows 4 such experiments using dosages which gave, as the legend shows, enormous secretions of pancreatic juice, with no hypoglycemia. On examining fraction B (residue) it was found to possess very little secretagogue activity and not to be hypotensive but to greatly depress the blood sugar. Figure 3 shows the results of two experiments. The blood sugar curve after crude secretin or the residue are quite similar.

Series III. Since Mellanby suggested the hypoglycemic properties of some secretin preparations may be due to included insulin, the point was investigated. Taking advantage of the fact that insulin is inactivated by pepsin-HCl, that method was used to see if insulin was present in crude

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Fig. 1. The effect of intravenous injection of crude secretin into normal dogs under chloralose.

1, 18 kilo male dog, 1 mgm. crude secretin per kilo, gave 4.2 cc. pancreatic juice in 20 minutes; *2*, 9 kilo female dog, 2 mgm. crude secretin per kilo, did not measure secretion; *3*, 11 kilo male dog, 1 mgm. crude secretin per kilo, 2.7 cc. pancreatic juice in 20 minutes; *4*, 8 kilo female dog, 2 mgm. crude secretin per kilo, 2.5 cc. pancreatic juice in 20 minutes.

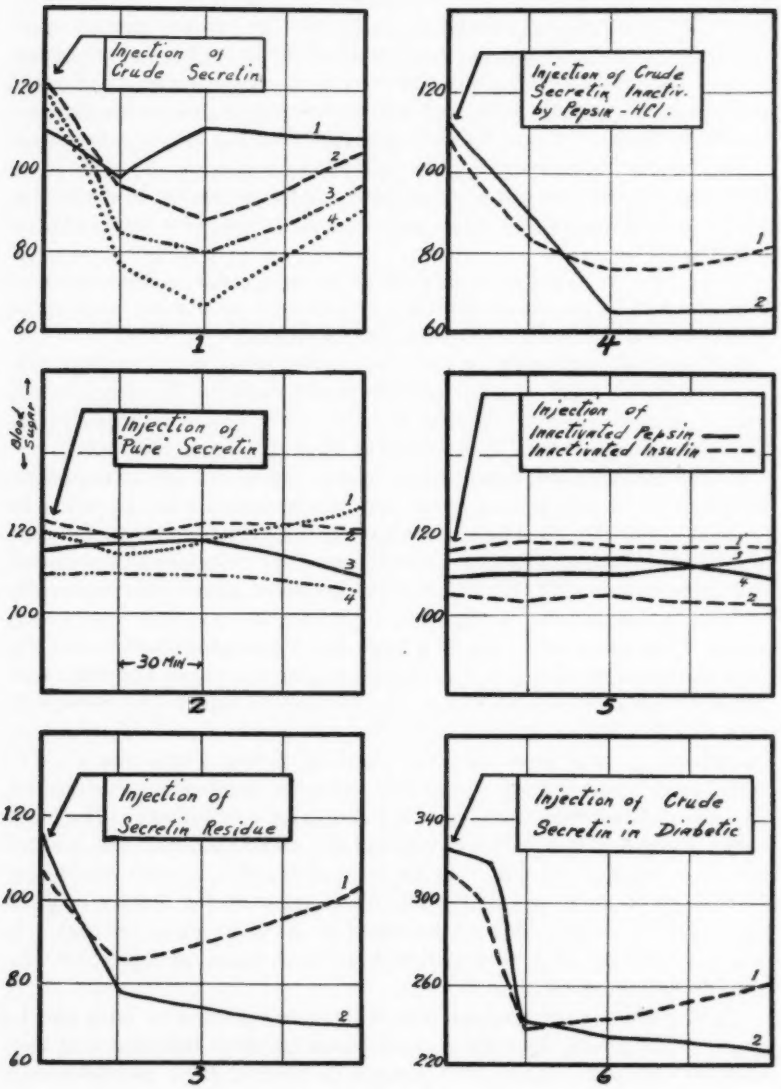
Fig. 2. The effects of intravenous injection of highly purified secretin on the blood sugar. *1*, 10 kilo male dog, 0.04 mgm. pure secretin per kilo, 4 cc. juice in 20 minutes; *2*, 10 kilo male dog, 0.09 mgm. pure secretin per kilo, 5.1 cc. juice in 20 minutes; *3*, 9 kilo male dog, 0.23 mgm. pure secretin per kilo, 7 cc. juice in 20 minutes; *4*, 9 kilo male dog, 0.22 mgm. pure secretin per kilo, 8.2 cc. juice in 20 minutes.

Fig. 3. The effect of intravenous injection of secretin residue on the blood sugar. *1*, 11 kilo female dog, 1½ mgm. residue per kilo, 1 cc. juice in 90 minutes; *2*, 11 kilo male dog, 3 mgm. residue per kilo, 0.8 cc. juice in 90 minutes.

Fig. 4. Effects of intravenous injection of inactivated crude secretin on blood sugar. *1*, 21 kilo male dog, 0.9 mgm. crude secretin per kilo inactivated by pepsin per kilo, only 0.4 cc. juice; *2*, 9 kilo male dog, 1 mgm. crude secretin per kilo inactivated by pepsin per kilo, only 0.6 cc. juice.

Fig. 5. Pepsin and insulin controls for experiments in figure 4. *1* and *2*, effect of intravenous injection of 10 units of insulin inactivated by pepsin or the blood sugar. *3* and *4*, effects of intravenous injection of 5 mgm. of inactivated pepsin on the blood sugar.

Fig. 6. The effects of intravenous injection of crude secretin in totally depancreatized dogs.


Injection of Crude Secretin
120
100
80
60
1
2
3
4
1
Injection of Crude Secretin Inactiv. by Pepsin-HCl.
120
100
80
60
1
2
4
Blood Sugar
Injection of Pure Secretin
120
100
1
2
3
4
←30 Min→
2
Injection of Inactivated Pepsin
Inactivated Insulin
120
100
1
3
4
2
5
Injection of Secretin Residue
120
100
80
60
1
2
3
Injection of Crude Secretin in Diabetic
340
300
260
220
1
2
6

Figs. 1 to 6

secretin. Large doses of crude secretin were incubated for 10 minutes with pepsin-HCl, neutralized, heated to inactivate the pepsin, and injected. The secretagogue activity was destroyed but the hypoglycemia characteristic of the crude secretin was observed as shown in figure 4. Control experiments were made using heat inactivated pepsin alone or insulin inactivated by pepsin. Figure 5 shows these experiments. These experiments show that the hypoglycemia which follows the intravenous injection of insufficiently purified secretin is not due to included insulin but to some other substance which is not heat labile and is not inactivated by pepsin-HCl as is insulin.

Series IV. It would be of interest to know if crude secretin produces hypoglycemia by increasing insulin secretion or by some other mechanism. This point was studied by two groups of experiments.

1. Dogs rendered totally diabetic some days before were injected intravenously with 2 mgm. of crude secretin per kilo and the blood sugar studied. Figure 6 shows the results obtained from two such experiments. This is in agreement with the reported findings of Takacs (1927–1928).

2. The question still remained as to whether or not all of the effects were due to the preparation *per se*, for diabetic animals are known to be especially sensitive to substances affecting the carbohydrate metabolism and because Zunz and La Barre (1929) showed by transfusion experiments that secretin increased the output of insulin which largely was responsible for the hypoglycemia. Using their technique the question was investigated. The pancreatic vein of a large dog A was anastomosed with the jugular vein of small dog B. A clamp was placed between the dogs, samples of blood taken and 2 to 4 mgm. of *crude secretin* injected intravenously into dog A. Three minutes later the clamp was removed permitting transfusion to take place from the pancreas of dog A into dog B. The transfusion continued for 15 minutes, then the anastomosis was severed. Samples of blood were taken each 30 minutes for 3 or 4 hours. The blood sugar of dog A (donor, being physiologically depancreatized) was lowered by 10 to 20 mgm. per cent. In the normal dog the decrease would have been 30 to 60 mgm. per cent. The blood sugar of dog B (receiving the pancreatic blood from dog A) decreased by 20 to 30 mgm. per cent. In some experiments dogs were used with adrenals intact, and in others with the adrenals removed.

These results agree, qualitatively, with those reported by Zunz and La Barre. The greater hypoglycemia observed by them indicates that their secretin was more impure with respect to freedom from physiologically active substances than the crude secretin used in these experiments.

It seems then that the mechanism of the hypoglycemia is due to at least two known factors: 1. The crude secretin *per se* diminishes the blood sugar as shown by the effects on the diabetic dogs. 2. The crude secretin augments the secretion of insulin, as shown by the transfusion experiments.

CONCLUSIONS

1. Crude secretin may be prepared which is not hypotensive, possesses great potency as a pancreatic secretagogue and contains varying amounts of hypoglycemic substances.

2. Crude secretin may be fractioned into one part which is only secretagogue and another which will lower the blood sugar.

3. The hypoglycemic fraction from crude secretin is not hypotensive, is not insulin, and produces hypoglycemia by at least two known factors—1, stimulation of secretin of insulin (most important); and 2, effects of the preparation *per se*.

4. Purified secretin has no effect on the blood sugar.

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THE EFFECTS OF STROPHANTHIN AND OF QUINIDINE ON THE RATE OF FIBRILLATION OF THE TONGUE FOLLOWING HYPOGLOSSOTOMY

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Since the experimental work of Mayer (1) upon contractile tissue cut from the disc of a jelly fish, the heart alone has furnished the basis for most of the experimental work bearing upon the problem of fibrillation. It occurred to us that a study of another organ, the tongue, might bring forth facts of interest to the general problem of abnormal cardiac physiology; for like the heart the tongue is possessed of both autonomicity and the phenomenon of fibrillation. In addition to having the mechanism of continuous fibrillation reside within its structure, the organ has a complex architecture which may resemble that of the heart in more than one way; for besides having fibers disposed of in three planes arranged so that bundles cross one another at right angles, there are many interlacing or branched fibers (2, 3). The organ, therefore, appears unique and satisfies certain physiologic and morphologic requirements for its comparison with the heart.

A simple mode of attack that suggested itself was an investigation of the response of the fibrillatng tongue to strophanthin and to quinidine. This, it was thought, would serve as a means of further elucidating the pharmacodynamic action of these drugs.

METHOD. Unilateral hypoglossotomy was performed on eight adult dogs under ether anesthesia. The nerve was cut across in the neck just above the ansa hypoglossus. Between nine and fifty-four days after this operation, electrograms were taken of the fibrillating side of the tongue. Barbital-sodium as a form of anesthesia was injected intramuscularly in amounts between 120 and 150 mgm. per kilo following the routine administration of 0.015 gram of morphine sulphate. The jaw of the animal was widely wedged open, and the tongue allowed to hang free in the mouth,

[1] Aided by the Emil and Fanny Wedeles Fund of the Michael Reese Hospital for the Study of Diseases of the Heart and Circulation, and in part by a grant from the Douglas Smith Foundation of the University of Chicago.

ventral side up. Electrograms were made three to five hours after the injection.

Non-polarizable electrodes were placed several millimeters apart near the tip of the fibrillating side of the tongue, because this part shows the most pronouced fibrillary movements and possibly the part least influenced by respiration. The electrodes were connected with a string galvanometer, operated with a loose string, one millivolt of current introduced with the tongue in the circuit causing a deflection of between 2.5 and 3.5 cm. An attempt was made to keep the degree of the deflection constant and the electrodes at fixed points.

The femoral vein was cannulated for injection purposes. Three animals received two 1 mgm. injections of strophanthin within thirty minutes; a fourth one received three such injections. Merck's ampoule preparation of strophanthin-K (Boehringer) containing 1 cc. of a sterilized 1:1000 solution was used. Before its intravenous injection, it was diluted up to 20 cc. with distilled water. An aqueous solution, (5 mgm. to 1 cc.) of quinidine sulphate (Mallinckrodt) was employed. Two animals received two injections of 0.060 gram within thirty minutes; another two received twice this dose (0.120 gram). The injection time was between 30 and 40 seconds.

Results. Unilateral section of the hypoglossal nerve produces continuous fine flickering or tremulous movements of the tongue on the ipsolateral side as early as the second or third day. As a result of unilateral motor paralysis, the tongue tends to deviate toward the paralyzed side, or to assume somewhat of an S-shape.

The effect of strophanthin and quinidine may best be seen by examining tables 1 and 2, and figures 1, 2 and 3. In three of the four experiments, a single injection of 0.060 gram of quinidine sulphate produced an average decrease of 34 per cent in the rate of oscillation before the end of the injection period; after this the rate became fairly progressively slower, and the subsequent injection had less influence on the rate of oscillation. At no time after the initial injection did the rate of oscillation exceed that of the control. The lethal dose of quinidine sulphate did not extinguish the fibrillary movement until respiration ceased. Shortly after the initial dose the process appeared slightly less active. Although no oscillations were recorded in the electrogram at the end of experiment 4, there was slight visible fibrillation.

The effect of intravenous strophanthin was the reverse of that of quinidine. The fibrillary movement visibly became more vigorous, and in all of the experiments in which this drug was employed there was a conspicuous increase in the rate of oscillation in the electrogram. In two experiments the rate was approximately double, within 6 and 13 minutes after 1 mgm. was administered. After the excitory effect wore off, there was a tendency to assume the rate prior to injection. Within thirty minutes, the

rate of oscillation approximated or slightly exceeded that of the control. In only one experiment (4) was the fibrillary movement terminated,

TABLE 1

Effect of quinidine sulphate on the rate of oscillation

DAYS AFTER HYPOGLOSSOTOMY	EXPERIMENT I 27 DAYS	EXPERIMENT II 27 DAYS	EXPERIMENT III 34 DAYS	EXPERIMENT IV 54 DAYS
Control rate	540–540	420–500	320–360–380	300–320
During injection	Q. S. 0.060 g.	Q. S. 0.060 g.	Q. S. 0.120 g.	Q. S. 0.120 g.
First 10 seconds		460	280?	220
Second 10 seconds		420	320	280
Third 10 seconds		220–280	?	240
Fourth 10 seconds	380	220	?	
Following Q. S.				
Min. 1	420–420	300	280	
2				
4	340–360	100–120		100
5	180–240			
6			250	
7			*0.120 g. Q.S.	
8	*Q.S. 0.060 g.		240	
9	200–220			
10		180–220		
11		*Q.S. 0.060 g.		160
13		120–240		
14				0†
16		160		
18		*Q.S. 0.060 g.		
19			260	
20			*Q.S. 0.120 g. 380 during injection	
21			300	
22		160	260	
23			220	
24			220	
29			240	

* Denotes subsequent injection.

† Although no oscillations are recorded in the electrogram there was slight visible fibrillation.

following the administration of a definitely lethal dose of strophanthin and when there was marked medullary depression.

COMMENT. The physiology of tongue fibrillation is but little understood and the site of action of strophanthin and of quinidine of the fibrillating

TABLE 2

Effect of strophanthin on rate of oscillation

DAYS AFTER HYPOGLOSSOTOMY	EXPERIMENT I 9 DAYS	EXPERIMENT II 22 DAYS	EXPERIMENT III 22 DAYS	EXPERIMENT IV 28 DAYS
Control rate	330	220–360	400–360	285–360
During injection	S. 0.001 g.	S. 0.001 g.	S. 0.001 g.	S. 0.001 g.
First 10 seconds				
Second 10 seconds				450–330
Third 10 seconds			300	540–330
Fourth 10 seconds			360	360–480
Following S.				
Min. 1			300	330–360
2			260	340–340
3		380–300		
4			320–280	480–420
6	600		420–380	300–330
8	640–620			420–420
9		220	260–300	
11				220
12	440–400			
13		600–540		260
15				320
16	330–336		*S. 0.001 g.	
17			240	360
18	312–330			
20			?	260
21	300–318			
23				300
24				240* S. 0.001 g.
25				360–480
26	260–280	260		
27	280 *S 0.001 g.			
30	100			†0
31	200			
33	340			
37			280	

* Denotes subsequent injection.

† Singularly enough at the end of this experiment the injection of intravenous ether produced fibrillation on the contralateral side of the tongue only, and this was of a very coarse grade.

tongue has not been demonstrated.[2] A consideration of circus movement as the underlying cause of tongue fibrillation becomes inevitable, although it is now inappropriate for us to consider this at length. If ventricular fibrillation may be considered a manifestation of circus movement, it is conceivable that this is the underlying mechanism for tongue fibrillation. Garrey (4) made the suggestion that circus movement, once started, might exist in a flat sheet of muscle without a central aperture. Theoretically the idea is conceivable to Lewis (5), although it is difficult for him to see how simple circus movement could continue regularly.

It is of interest to note that the rate of fibrillation in the tongue following hypoglossotomy is approximately that of the auricle in which the phenomenon occurs, and also that the effects of strophanthin and of quinidine on the rate of oscillation are quite parallel in the two structures. The change in the rate of oscillation of the fibrillating auricle is thought to be a direct one (at least chiefly so) with quinidine sulphate; but largely an indirect one with strophanthin. Here, quinidine terminates the process frequently, strophanthin rarely. In the tongue, quinidine does not terminate the process in the hypoglossotomized animal in the acute experiment although strophanthin occasionally produces (4) a transient marked fibrillation in the narcotized but intact animal. These are arguments neither for nor against the theory of circus motions as applied to the tongue.

[2] Fibrillation of the tongue has persisted following section of the hypoglossal nerve after subsequent section of the seventh, ninth, tenth and eleventh cranial nerves and also the cervical sympathetics. Personal communication from Dr. Lewis Pollock and Dr. Loyal E. Davis.

Fig. 1. Showing the effects of quinidine sulphate on the rate of oscillation of the fibrillating tongue. (Expt. 2.)

A. Control. Rate 420 to 500 per minute.

B. End of the injection period in which 0.060 gram of quinidine sulphate was given intravenously. Rate 220 to 280 per minute.

C. Twenty-two minutes after the initial injection and 1 minute after the third injection of quinidine sulphate (total of 0.0360 gram). Rate approximately 100.

Fig. 2. The effects of strophanthin on the rate of oscillation of the fibrillating tongue. (Expt. 1.)

A. Control rate 330 per minute.

B. Eight minutes after intravenous injection of 0.001 gram strophanthin K. Rate 620 to 640 per minute.

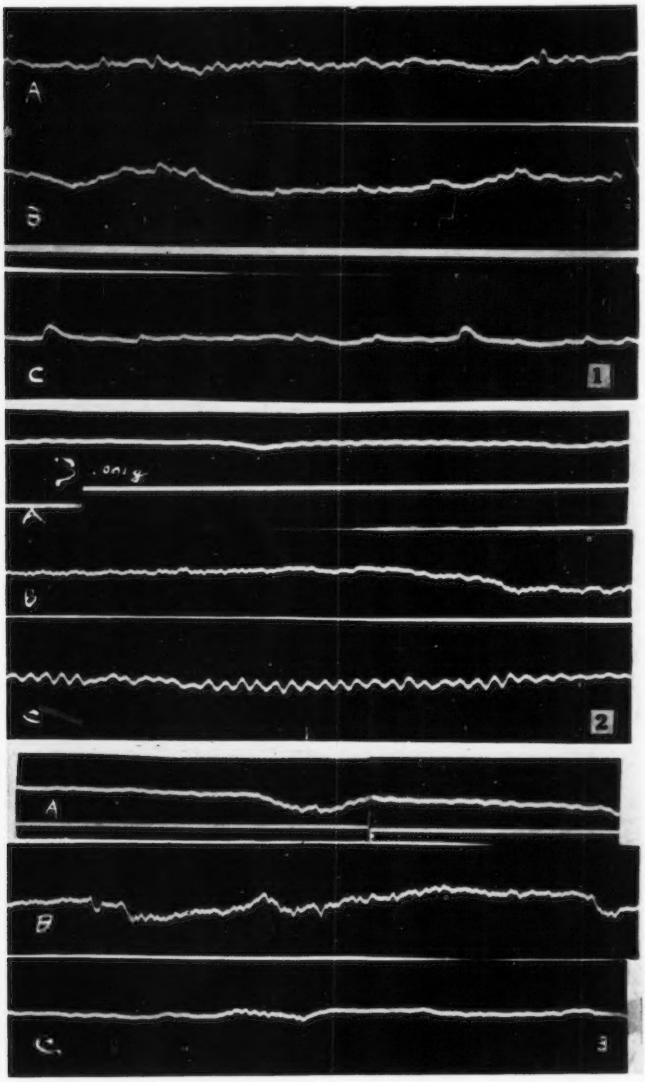
C. Eighteen minutes after intravenous injection of 0.001 gram strophanthin K. Rate 312 to 330 per minute.

Fig. 3. Showing the effect of strophanthin on the rate of oscillation of the fibrillating tongue. (Expt. 2.)

A. Control rate 220 to 360 per minute.

B. Thirteen minutes after the intravenous injection of 0.001 gram strophanthin K. Rate 540 to 600 per minute.

C. Twenty-seven minutes after the intravenous injection of 0.001 gram strophanthin K. Rate 260 per minute.



Figs. 1 to 3

SUMMARY

The rate of oscillation of the fibrillating tongue following hypoglossotomy is approximately that of fibrillating auricle.

Quinidine sulphate produces a marked slowing of the rate of oscillation of the fibrillating tongue; strophanthin greatly augments it.

I am greatly indebted to Dr. Lewis Pollock for showing much interest in this research and for suggesting the electrical method of recording fibrillary movements of the tongue; also to Dr. Anton J. Carlson under whose guidance this research was conducted.

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BASAL METABOLISM IN ORIENTALS

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The basis of the discussion of regional variations of basal metabolism has shifted from a climatic to a racial one since Benedict and his collaborators (1) showed that the basal metabolic rate of a group of oriental students living for a considerable time in this country under absolutely western conditions was distinctly below western standards. The Chinese Physiological Society has organized research on this question and a large number of results (2) indicates that there is a low basal metabolic rate in Chinese (climatic influences may be superimposed) (3). In one group of experiments, however, (2) Necheles and Shen showed values that are about 5 per cent higher than the rest. In this first group, the body surface was determined by linear measurements. Stevenson (4) and Necheles and Shen (3) have shown that the computation of the body surface in Chinese persons with the height weight formula (Aub, Du Bois), gives about 5 per cent higher values than those computed by linear measurements. There is thus evidence that the basal metabolic rate of Chinese may be about 5 per cent too low if the height weight formula is used. The above findings are contradictory to those of Benedict (1) and Wadell (5).

During a prolonged stay in China the writer was impressed by the observation that the average Chinese is always more relaxed than the average Westerner; the vivid Southern Chinese as well as the more quiet Northern Chinese. Their muscular tone seemed to be constantly lower than that of Westerners and it was conjectured that this might be the reason of their lower basal metabolic rate. Searching for a method by which to put this hypothesis to test the writer was advised by Dr. F. G. Benedict[2] to make use of the difference in basal metabolic rate between waking and sleeping. If no difference were found the indication would be that complete relaxation already existed in the waking state.

A respiration chamber[3] was constructed which was large enough (1060 L.) to prevent feelings of oppression and to make the subjects feel at ease.

[1] Work done while holding a fellowship of the Rockefeller Foundation.

[2] To whom the writer wishes to express his gratitude.

[3] The method will be described in full in connection with a later report on additional data.

The author's work included nine experiments on eight oriental subjects and two experiments on two Western subjects. The subjects slept in the chamber for the whole night and their basal metabolic rate was determined during and after sleep. The temperature in the chamber was kept constant by water flowing through lead pipes; O_2 only was determined.

The average of the basal metabolic rate of the first seven experiments (subjects 1 to 6, table 1) is, for waking −12.3 per cent (Aub, Du Bois' standards) and −10.1 per cent (Harris, Benedict standards); for sleeping

TABLE 1

Basal metabolism during sleep and wake in Orientals

SUBJECT NO.	RACE AND SEX	YEARS IN U. S. A.	AGE	NUDE WEIGHT	HEIGHT	PER CENT DEVIATION FROM STANDARDS					
						Aub Du Bois		Harris Benedict		Difference between sleeping and waking	
						Sleeping	Waking	Sleeping	Waking	Aub Du Bois	Harris Benedict
			years	*kgm.*	*cm.*						
1	Jap. m.	5	27	45	160	−18.7	−17.7	−15.1	−13.4	−1.0	−1.7
						−14.9	−8.4	−10.9	−3.9	−6.5	−7.0
2	Chin. m.	9	22	47	161.5	−14.9	−13.9	−13.3	−12.2	−1.0	−1.1
3	Chin. m.	1	24	56.5	172.8	−13.9	−12.9	−13.0	−10.7	−1.0	−2.3
4	Filip. m.	2	19	46.1	157.5	−13.6	−6.6	−8.7	−11.4	−7.0	−2.7
5	Korean m.	14	36	67	170	−17.0	−11.1	−14.3	−6.4	−5.9	−7.9
6	Chin. m.	6	25	45	163.2	−21.0	−15.3	−18.2	−12.4	−5.7	−5.8
7	Filip. m.	5	24	53	161.6	−15.9	−3.3	−14.5	−1.5	−12.6	−13.0
8	Filip. f.	2	26	45.7	150.3	+6.6	+24.0	+2.5	+21.8	−17.4	−19.3
			BASAL METABOLISM DURING SLEEP AND WAKE IN WESTERNERS								
9	Italian* m.		40	59.86	170.7	−13.5	+4.8	−7.6	+11.7	−18.3	−19.3
10	Am. Ital. m.		21	71	169.4	−1.8	+11.9‡	−3.2	+10.0	−13.7	−13.2†
11	Am. Engl. m.		19	70	171.5	−2.9	+24.6‡	−0.9	+27.1	−27.5	−28.0†

* Levanzin, first day of starvation (8).

† Moved during sleep.

‡ Athletic.

−14.9 per cent and −13.4 per cent respectively; there is a difference of only 2.6 and 3.3 respectively between sleeping and waking. The last two experiments (subjects 7 and 8), with a basal metabolic rate at and above normal during waking, have respectively a difference between sleeping and waking of 12.6 or 13.0 and 17.4 or 19.3.

In adult Westerners Loewy (6) and Johanson (7) found practically no difference between waking and sleeping; their subjects were adept at relaxation, however (see 8, p. 351, and 6, morphine sleep). Pettenkofer

and Voit (9) found a 28 per cent increase of CO_2 excretion following sleep; Benedict (8) a 21 per cent increase of O_2 consumption. The very complete data of Benedict (subject 9) and data from two more experiments by the writer (subjects 10 and 11) have been added to table 1. The basal metabolic rate of Benedict's subject was slightly above normal while waking and −18.3 or −19.5 below the standards during sleep; i.e., during sleep, this subject's basal metabolic rate was within the range of that of the first group of Orientals. The two experiments of the writer (subjects 10 and 11) show a very marked difference between sleeping and waking. The basal metabolic rate of these subjects is above the standards but their basal metabolic rate during sleep is too high due to bodily movements.

CONCLUSIONS AND SUMMARY

Six Orientals with a low basal metabolic rate did not show any appreciable drop in basal metabolic rate during sleep.

Two Orientals with a normal or high basal metabolic rate showed a considerable drop in basal metabolic rate during sleep.

Westerners with normal and high basal metabolic rates showed a drop during sleep comparable to that of the second group of Orientals.

There is evidence that the lower basal metabolic rate of Orientals is partly due to a greater degree of constant relaxation.

The writer wishes to express his deepest gratitude to Dr. A. J. Carlson for hospitality and encouragement.

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THE USE OF CONSTANT GLUCOSE INJECTIONS FOR THE STUDY OF INDUCED VARIATIONS IN CARBOHYDRATE METABOLISM

I. Methods and Controls

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The investigations to be reported in the succeeding papers required a method capable of detecting a depression or suppression of the normal ability to utilize glucose in acute experiments on unconscious animals. The careful studies of Woodyatt, Wilder, Sansum and later Felsher (1) show that normal animals of various species respond in a characteristic manner to the constant continuous administration of glucose by vein at uniform rates. Their findings, insofar as they have been tested, have been confirmed in all essential respects (2), (3). It appeared that an application of their methods and principles would fulfill the requirements of the proposed investigation.

Among other things the following facts are known as a result of the studies in Woodyatt's laboratory. If a resting, calm, normal rabbit, dog or human receives glucose by vein at a uniform rate over a period of several hours, the appearance and degree of glycosuria will be determined, within moderate limits, by the quantity of glucose given for each unit of body weight for each unit of time (1a, b). Expressed for convenience in terms of grams of glucose per kilogram body weight per hour, the *rate* of administration, so defined, will determine the response to the injection and not to any great extent the concentration of the injected solution or the total dose. Thus, with advancing administration rates, the excretion of reducing substances does not vary widely from that of fasting until the injection rate exceeds on the average about 1 gram per kilogram per hour, the actual level being slightly variable in different animals. With any given rate of injection above this level, abnormal glycosuria begins within the first hour and after an interval of one to four or more hours becomes virtually constant for an indefinite time (1b, c, d, f). Upon passing the critical level with any animal, advancing rates of administration lead first to an abrupt acceleration of the rate of excretion for a time, followed by a

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somewhat more gradual but progressive increasing glycosuria until at high rates, such as 7 grams per kgm. per hour, the excretion approaches but does not exceed 35 to 40 per cent of the supply (1b, h).

This response varies somewhat in different animals and species and it may be modified to a limited extent by variable feeding conditions before the injection, but on the whole is characteristic within a moderate range, and especially under constant conditions.

These data might be expressed differently as follows. When glucose is administered by vein at uniform rates up to approximately 1 gram per kgm. per hour practically all of it is retained no matter how long administered. At any higher rate, after a transition period required for equilibrium, a fixed portion of it is retained, and with advancing supply rates the absolute retention increases. Woodyatt considers, justifiably it seems, that on reasonable grounds it does not require proof to assume that the retained sugar represents the total utilization, taken as the combined oxidation and storage as glycogen and fat. He says, " . . . . the tendency is toward the establishment of an equilibrium which is manifested by a constancy of the rate at which the organism rids itself of the substance as such, by excretion, or chemical change, or both. The rate of entry is then equalled by the rate of utilization plus the rate of elimination" (1d, p. 355). The third paper of this series contains reports of experiments in which simultaneous respiratory and urinary observations provide confirmatory data for this contention.

If it is conceded that with a constant supply of glucose exceeding the maximum utilization rate, changes in the utilization rate will be reflected, after a preliminary period, as reciprocal changes in the excretion rate, then the method may be adapted admirably for the study of experimentally induced alterations of carbohydrate metabolism, because the total utilization of sugar may be measured quantitatively, hour after hour, as the difference between the supply and the excretion. Experiments of this nature have been undertaken by the present writer and will be reported in this and the three following communications.

Many of the experimental procedures involved in such an investigation are technically difficult or impossible in conscious animals. Hence, with a choice between anesthesia, decerebration and curare, the first was selected as the least objectionable. It should be emphasized that at no time have any illusions been entertained concerning the harmlessness of the anesthesia. From the beginning it has been considered objectionable but necessary, and its complicating possibilities have been overvaluated, if anything, in interpretation. As a matter of fact, however, it has transpired that satisfactorily even physiological states can be maintained for many hours under uniform anesthesia, and with experience uniformity of the

anesthesia can be produced and judged easily. Furthermore, even though the anesthesia is admittedly objectionable, from another standpoint it is advantageous because it eliminates emotional factors and muscular activity entirely. It seems debatable which of the disturbing sets of conditions would be more difficult to control in experiments of this sort.

To begin with, therefore, it was necessary to establish the response of the anesthetized animal to the continuous intravenous injection of glucose at a constant rate. A similar study has been made in dogs by Hines, Boyd and Leese (4) with results identical, insofar as they are comparable, with those to be reported here.

METHODS AND MATERIALS. Healthy adult female cats weighing from 2 to 3.5 kgm. were used exclusively. Except when otherwise noted, they ate salmon, lean meat and milk until 24 hours before the start of the experiment when they were deprived of food but allowed water.

Amytal (iso-amyl-ethyl-barbituric acid, Lilly) permits the maintenance of a remarkably stable physiological condition under adequate surgical anesthesia for hours. In proper dosage its effect on the blood-sugar concentration is negligible, the arterial pressure, heart frequency and respiratory rate remain within normal limits after the first hour or so and the rate of metabolism, while slightly below the normal range, is constant for hours unless the degree of anesthesia changes perceptibly (5), (16). These observations have been amply confirmed by the present writer. Amytal was selected, therefore, as the anesthetic of choice.

It was given intraperitoneally, subcutaneously, or both together as the sodium salt. The most satisfactory initial dosage was usually 0.06 to 0.07 gram per kgm., resulting in apparently uniform anesthesia for 4 to 10 hours. Small second doses were required occasionally. Shivering invariably occurred as a sign of lightening anesthesia, preceded by increased respiratory rate, exaggerated reflexes and stiffening of the extremities when handled.

It became apparent after the first few experiments that additional dosage of amytal was required to insure uniform anesthesia for a sufficent length of time to permit the desired investigations. Since every experiment involved the intravenous injection of a solution at a constant rate anyway, a small amount of amytal was incorporated in each solution and hence administered by vein at a constant rate throughout the experiment. When given at a rate of about 3 mgm. per kgm. per hour this procedure permits the maintenance of a surprisingly even anesthesia for as long as two days, or at least ample time for the satisfactory conclusion of most of the experiments.

Usually within an hour of the onset of the anesthesia the animal was placed on a board provided with a heating pad and catheterized. Female cats may be catheterized easily by means of a tube of glass, metal or fairly

stiff rubber about 10 cm. long and 2 mm. in diameter; closed, smoothly rounded and perforated on the side at the bladder end. The external urethral orifice on the ventral wall of the vaginal canal may be brought into view with an ordinary nasal speculum. With the bladder empty the injection was started and continued uniformly throughout the experiment, by means of a cannula tied into a saphenous vein. The injecting apparatus has been described elsewhere (6): it is sufficient to state here that it ordinarily performed steadily, delivering constant volumes hour after hour within limits of 1 per cent, which is considered negligible under the conditions. The actual delivery was checked by means of hourly burette readings in each experiment. Any interruption of the injection was considered cause for rejection of findings.

The urine was collected continuously during the experiment through the retained catheter. At intervals, usually hourly, it was separated into single specimens with care that at the end of each period the bladder was completely empty. A little experience with this procedure enables one to judge accurately when scarcely a drop of urine remains in the bladder. After noting the volume of urine each specimen was made up to a standard volume, usually 10 or 20 cc. and analyzed for sugar according to the method of Folin and Berglund (7).

Blood was drawn hourly in most cases, in 0.1 cc. samples from a slit in the ear, and its sugar concentration determined by means of the ferricyanide method of Folin (8). In experiments in which the oxygen consumption was determined a tracheal cannula was inserted at or before the beginning of the experiment and the oxygen absorption measured in a closed circuit of the Benedict-Roth type (9), equipped with metal flutter-valves and Wilson's soda-lime.

Dextrose made by the Corn Products Refining Company was used for all glucose injections. Compared with U. S. Bureau of Standards samples, its iron and copper reducing power, melting point and rotatory values indicated it to contain up to 5 per cent water and to be pure otherwise. It was given in 3.6 to 28.7 per cent concentrations, but for the most part approximately 10. Each solution was analyzed simultaneously with the urine specimens for the corresponding experiment by the same method.

During each experiment the animal was covered and its temperature maintained between 37.5 and 38.5°C. by means of the electric heating pad. No aseptic precautions were considered necessary because of the comparatively short nature of the experiments. With but a few exceptions, repeated experiments were never performed on the same animal.

Only data obtained under the conditions defined above were considered acceptable. Findings were rejected if the anesthesia was too deep (absent reflexes, slow respiration and slow and feeble heart action, cold extremities, sluggish blood flow) or too light (rapid breathing and heart rate, exag-

gerated reflexes, stiffness, shivering, increasing temperature). Other irregular conditions considered to be cause for rejection of findings included demonstrable pregnancy, interruption of the glucose administration and occasional difficulty in making accurate urine collections due to intravesical bleeding or anomalous urinary passages.

RESULTS. *Relation between rates of administration and excretion of sugar.* Taken individually, there is considerable variation in the response of different animals to the same rate of administration. Chart 1 is a composite representation of the quantitative relation between the excretion and administration in all the uncomplicated experiments providing such

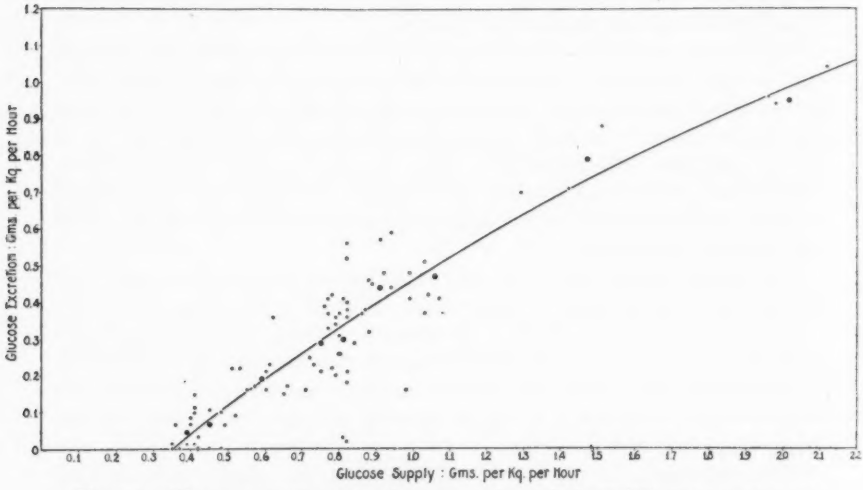

Chart 1. Glucose excretion at various rates of supply in 82 different experiments with amytalized cats. The circles represent the average excretion for 2 to 10 hours (after comparative constancy was established) in individual experiments, the solid dots the averages for groups of experiments, and the curve the approximate mean for the entire series. All values are expressed in grams per kilogram per hour.

data. Each individual circle represents the average excretion (after comparative constancy was established) for 2 to 10 hours, at the administration rate indicated, both values expressed as grams per kgm. per hour. Thus, for example, in the group of experiments with injection rates of 0.7 to 0.8 gram per kgm. hour, the excretion ranged between 0.16 and 0.42 gram per kgm. hour, or 23 and 54 per cent of the supply. Similar variations exist in the other groups.

Taken collectively, however, a definite relationship between the supply and excretion is apparent. If the experiments are divided into groups according to rates of supply, the averages for the groups show that the

excretion increases with the supply as indicated by the solid dots and curve in chart 1. In general, this response is similar to that observed by Woodyatt in normal animals insofar as the absolute and relative excretion increases with advancing injection rates. The slope of the curve suggests the excretion of approximately 50 per cent of the supply at administration rates in excess of 1.5 grams per kgm. hour.

It is apparent, therefore, that although the response of any given animal under these conditions can be predicted only within limits of accuracy of approximately ±15 per cent, abnormal glycosuria will appear when glucose is supplied at rates exceeding, on the average, about 0.35 gram per kgm. per hour, and also that as the supply is increased above this level the excretion also increases but appears not to be capable of exceeding one-half the supply no matter how great the supply.

It is not possible to establish a similar correlation between the blood-sugar concentration and the rate of supply with the data at hand. Although in general the highest blood sugar occurred with the greatest supply rates and vice versa, there were so many exceptions both at low and high rates that a quantitative relationship even on the basis of averages is not apparent. As an extreme example, two different animals with blood-sugar concentrations of 0.3 per cent excreted 30 and 700 mgm. per kgm. per hour respectively. Boyd, Hines and Leese (2) found the same to be true in normal dogs, an observation which indicates that the variability of the resulting glycemic concentration in different animals receiving sugar at identical rates in the present research cannot be attributed to the anesthesia. This variation between animals could be explained readily if a disproportion existed between the body weights and blood volumes (including fluids accessible for free diffusion) of different animals.

Thus, the general relationships between supply and excretion appear to hold for amytalized cats as for normal animals of other species and to differ only in the tolerance of the animals for glucose. There can be little doubt that the diminished tolerance is due to the anesthesia and not to the difference in species in view of the comparative findings of Hines, Boyd and Leese in amytalized and normal dogs (4). It is also possible that the anesthesia results in wider variations in the responses of individual animals to the injection. The available data on normal animals are inadequate to settle this point.

With this general background it is possible to take up the findings in individual cases. In order to avoid unnecessary repetition only one or two experiments illustrating each point will be reproduced. It should be understood, of course, that only results which have been adequately confirmed are reported as typical.

The constancy of the response in the same animal under uniform conditions. As long as the anesthesia is maintained uniformly the response

of any animal to the administration of glucose at any given rate is constant within moderate limits. The experiments reproduced in charts 2, 3 (table 1) and 4 are typical. With constant glucose supplies of different magnitudes the glycosuria by half-hours and the blood-sugar concentration by hours were virtually constant in each case for from 5 to 18 hours.

In experiment 37 (table 1) this constancy was maintained in spite of the fact that the urine volume varied from 1.6 to 14.8 cc. per half hour and the percentage of sugar dropped correspondingly from 10.8 to 1.33. Within rather wide limits the functional capacity of the kidneys does not appear to interfere seriously with the glucose excretion, as illustrated in experiment 49 (chart 5). Here the glycosuria fluctuated somewhat but

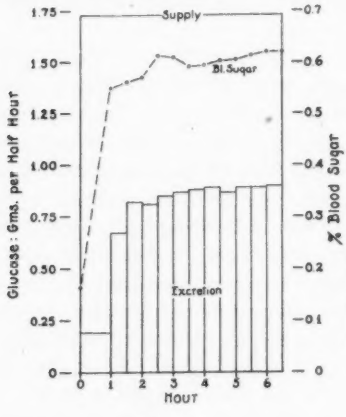

Chart 2

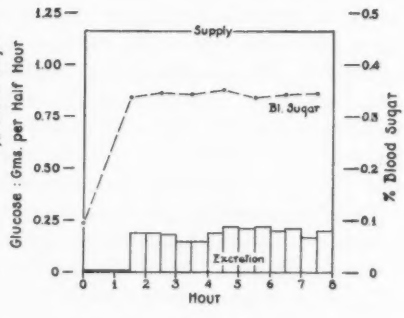

Chart 3

Chart 2. Experiment 7. Cat 231, weight 2.44 kgm. Glucose supply: 19.0 cc. 18.2 per cent solution or 3.46 grams per hour = 1.42 grams per kgm. per hour.

Chart 3. Experiment 37. See table 1.

the excretion of phenol-sulphone-phthalein, which was given at a constant rate along with the glucose, was perfectly constant in spite of variations in the urine volume within a range of 3.5 and 16.1 cc. per half-hour. Results of this character have verified the functional integrity of the kidney under these conditions and demonstrate that, when the exogenous supply is constant, variations in glycosuria are the consequence either of changes in the supply (from endogenous sources) or changes in the rate of utilization. Both of these circumstances are probably manifested when the anesthesia is uneven.

Repeated observations by various workers using different methods appear to have established the fact that the ability of the normal mammalian organism to utilize glucose is diminished by starvation and enhanced

by sugar administration. In the first place the hyperglycemic response to single doses of glucose and other carbohydrates is greatest and most prolonged after fasting and each of several successive doses results in a smaller response (10). Secondly, the hypoglycemic phase frequently observed following the hyperglycemia due to carbohydrate administration is likewise suggestive (11). Grafe and Meythaler have demonstrated hypoglycemia by the introduction of glucose directly into the pancreas circulation (12). Various attempts to estimate the rate of insulin secretion, although conducted by means of uncertain physiological methods,

TABLE 1

Urine and blood sugar findings with constant injection of glucose (See chart 3)

HOUR	BLOOD SUGAR*	URINE		
		Volume	Sugar	
			Per cent	Gram
	per cent	*cc.*		
0	0.095			
0 –1½	0.338	1.8	1.33	0.02
1½–2		2.0	9.30	0.19
2 –2½	0.347	1.8	10.8	0.19
2½–3		1.7	10.6	0.18
3 –3½	0.343	1.6	9.44	0.15
3½–4		1.7	8.71	0.15
4 –4½	0.352	2.9	6.69	0.19
4½–5		5.9	3.68	0.22
5 –5½	0.338	10.2	2.03	0.21
5½–6		14.6	1.49	0.22
6 –6½	0.343	13.2	1.55	0.20
6½–7		11.2	1.89	0.21
7 –7½	0.345	11.6	1.47	0.17
7½–8		14.8	1.33	0.20

Experiment 37. Cat 250, weight 2.37 kgm. Glucose supply 24.5 cc. 9.48 per cent solution or 2.33 grams per hour = 0.98 gram per kgm. per hour.

* Taken at end of period.

indicate consistently that the administration of glucose leads to increased pancreas activity and augmented insulin secretion (13) as might be expected. Finally, all investigators working with constant injection methods have noted a tendency for the blood sugar and glycosuria to reach a maximum, during the first hour or two of the injection, which is often followed by a recession of variable degree in the succeeding periods (1h, 2, 3). Jordan has shown that a preliminary injection of sugar invariably reduces the glycosuric response to a subsequent constant injection (3), and Boyd, Hines and Leese (2) and Thalhimer et al. (14) observed distinct hypoglycemia shortly after interrupting the injection.

The results of the present experiments are in accord with these findings. There was a distinct tendency for the blood and urine sugar to reach a

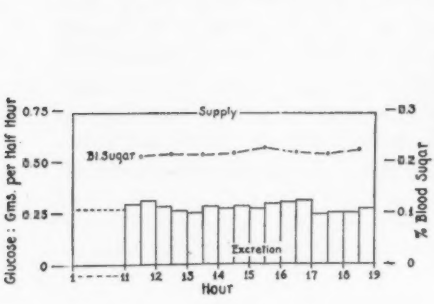

Chart 4

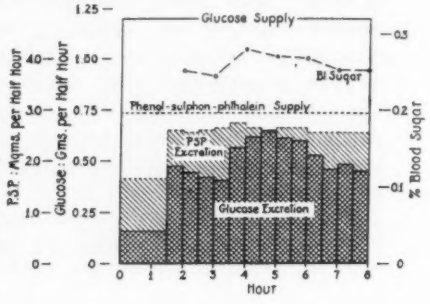

Chart 5

Chart 4. Experiment 43. Cat 257, weight 2.40 kgm. Glucose supply: 16.0 cc. 9.17 per cent solution or 1.47 grams per hour = 0.61 gram per kgm. per hour.

Chart 5. Experiment 49. Constant phenol-suphone-phthalein excretion simultaneously with irregular glucose excretion, showing the latter not to be due to impaired excretory efficiency. Cat 260, weight 2.79 kgm. Glucose supply: 24.4 cc. 9.85 per cent solution or 2.40 grams per hour = 0.86 gram per kgm. per hour. P-S-P supply: 24.4 cc. 0.024 per cent solution = 5.86 mgm. per hour.

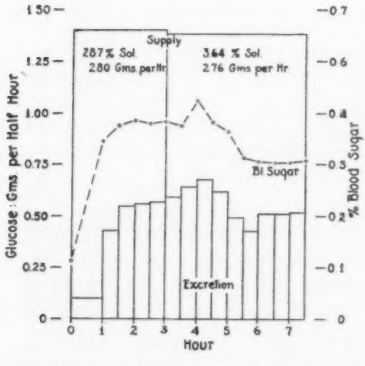

Chart 6. Experiment 12. See table 2.

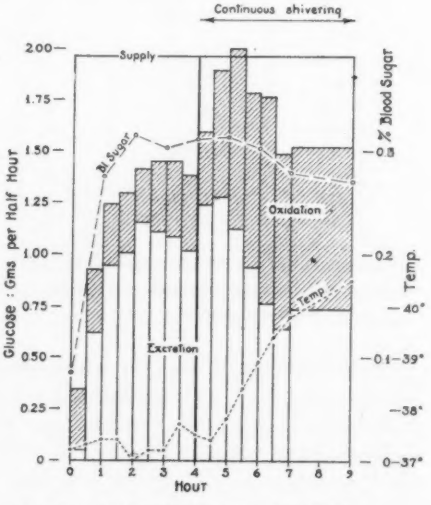

Chart 7. Experiment 52. See table 3.

maximum at the peak of the initial rise, i.e., during the second or third hour of the injection, decreasing somewhat thereafter, as a rule, but fol-

lowed later by a subsequent increase. This tendency may be noted in most of the experiments reproduced. The phenomenon is particularly noticeable with rates just high enough to cause a minimum abnormal glycosuria, as noted by Jordan. With greater absolute glucose excretions the phenomenon is obscured, partly because the transitional adjustment period is longer with the higher injection rates, and partly because the quantity of sugar involved in the phenomenon is usually insignificant in proportion to the degree of glycosuria. In any event, alterations occur-

TABLE 2

Influence of variation in the water supply with constant sugar supply (See chart 6)

HOUR	BLOOD SUGAR*	URINE			SUGAR ADMINISTRATION			
		Volume	Sugar		Cc. per hour	Per cent	Grams per hour	Grams per kgm. hour
			Per cent	Gram				
	per cent	*cc.*						
0	0.114							
0 –1	0.345	4.0	4.93	0.20				
1 –1½	0.377	6.2	6.90	0.43				
1½–2	0.387	7.4	7.47	0.55	9.77	28.7	2.80	1.04
2 –2½	0.380	7.6	7.36	0.56				
2½–3	0.385	7.9	7.25	0.57				
3 –3½	0.377	9.9	5.92	0.59				
3½–4	0.425	12.5	5.13	0.64				
4 –4½	0.382	20.0	3.40	0.68				
4½–5	0.364	21.8	2.86	0.62				
5 –5½	0.313	25.5	1.91	0.49	76.6	3.64	2.76	1.03
5½–6	0.306	17.9	2.42	0.43				
6 –6½	0.302	14.2	3.60	0.51				
6½–7	0.303	12.3	4.17	0.51				
7 –7½	0.308	12.8	4.44	0.52				

Experiment 12. Cat 238, weight 2.682 kgm. at start. Net gain in weight 160 grams. Net observed fluid retention 194 cc.

* Taken at end of period.

ring in this manner during the first hours of the experiment fall within limits of fair constancy, especially with higher injection rates.

Ordinarily the total excretion and blood concentration of sugar were roughly parallel with a given rate of administration, but not necessarily so. That they may proceed independently was demonstrated by Woodyatt and Sansum in an experiment in which the excretion was diminished by about 30 per cent in spite of a constant blood sugar (1f), and in two others in which conversely the glycosuria was sustained at constant levels in spite of as great as 50 per cent fluctuations in the blood sugar (1f, g). Experiment 12 (chart 6, table 2) of the present series duplicates the latter observa-

tions, providing additional evidence for the view expressed by Woodyatt (1g) that other factors besides concentration are important in determining the physiological effects of a substance in the blood plasma.

In contrast to its variability in different animals, however, under uniform conditions the blood sugar was maintained at a very constant level in any given experiment, tending to rise very slightly as a rule if at all variable.

TABLE 3

The effect of shivering on the excretion and estimated oxidation of sugar with the supply constant (See chart 7)

HOUR	TEMPERATURE	BLOOD SUGAR‡	URINE		OXYGEN CONSUMPTION		SUGAR OXIDATION*	SUGAR OXIDATION PLUS EXCRETION*	REMARKS
			Sugar	N	Total	Non-protein*			
	°C.	per cent	grams	mgm.	cc.	cc.	grams	grams	
0 – ½	37.2		0.57		530				Before injection
½–1	37.2	0.07	(mgm.)		609				
0 – ½	37.3		0.05		641	221	0.30	0.35	Inj. started
½–1	37.4	0.27	0.62		631	211	0.28	0.90	Quiet
1 –1½	37.4		0.95		635	215	0.29	1.24	Quiet
1½–2	37.0	0.31	1.01	397	637	217	0.29	1.30	Quiet
2 –2½	37.2		1.15		621	201	0.27	1.42	Quiet
2½–3	37.2	0.30	1.10		681	261	0.35	1.45	Quiet
3 –3½	37.7		1.08		698	278	0.37	1.45	Quiet
3½–4	37.5	0.31	1.02	227	679	259	0.35	1.37	Quiet
4 –4½	37.4		1.23		683	263	0.35	1.58	Shivering
4½–5	37.8	0.31	1.28		873	453	0.61	1.89	Shivering
5 –5½	38.4		1.12		1,078	658	0.88	2.00	Shivering
5½–6	38.9	0.30	0.94	276	1,040	620	0.83	1.77	Shivering
6 –6½	39.4		0.76		1,164	744	1.00	1.76	Shivering
6½–7	39.8	0.28	0.65		1,042	622	0.83	1.48	Shivering
7 –9	40.5	0.27	0.74**	379	1,005**	585**	0.78**	1.52**	Shivering

Experiment 52. Cat 263, weight 3.04 kgm. Glucose supply 38.2 cc. 10.3 per cent solution or 3.93 grams per hour = 1.29 grams per kgm. per hour.

* Based on the average nitrogen excretion for the entire experiment, and assuming an R. Q. of unity.

** Average per half-hour.

‡ Taken at end of period.

Indeed, experience with the method results in the distinct conclusion that in any experiment the blood sugar is less variable than the glycosuria and, inasmuch as it is less variable, *that it is less sensitive as an indicator of changes in the fate of the administered glucose* notwithstanding the effect of variable kidney factors on the glycosuria. This question will be considered more fully in the section devoted to experiences with epinephrine and insulin.

The response to increased muscular activity (shivering). As mentioned above, lightening of the anesthesia under amytal is attended first by beginning responsiveness to external stimuli and later by continuous shivering, always prolonged and violent if unchecked by further amytal administration. Early in the course of the work it was observed repeatedly that this phenomenon resulted first in increased and later decreased glycosuria, usually with corresponding but less marked changes in the blood-sugar concentration.

The oxygen consumption was measured simultaneously in a number of experiments, of which experiment 52 (table 3, chart 7) is representative. After the first two hours of adjustment the glycosuria was constant with the animal quiet, but first rose and then fell when shivering occurred. The total oxygen consumption was constant until shivering began, when it rose to nearly twice its original level. Without knowledge concerning the carbon dioxide elimination it is of course not possible to compute the glucose oxidation accurately. However, Hines, Boyd and Leese have found that similar injections of glucose into amytalized dogs led to R. Q.s within a range of 0.95 and 1.08, unless the anesthesia was profound (4). Similar observations in cats will be reported subsequently. Thus an approximate calculation is possible in experiment 52 based on the assumption that the respiratory quotient was unity throughout, and based also on the average nitrogen excretion for the entire period (on account of the obvious washing out during the first two hours). It indicates clearly that the glucose not appearing in the urine as shivering proceeded may be accounted for on the basis of increased oxidation, and that the sum of that excreted and oxidized is a constant proportion of the supply. The extra sugar appearing in the urine at the onset of shivering is not accounted for on this basis, of course. It seems reasonable to suppose that it originated in the most probable internal source, namely, glycogen. These calculations illustrate the possibilities of the method as well as the probable relations involved.

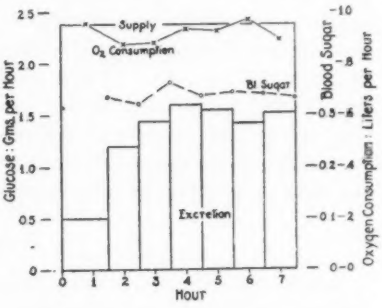

Chart 8. Experiment 59. Constant oxygen consumption, glucose excretion and blood sugar concentration under uniform anesthesia. Cat 272, weight 2.56 kgm. Glucose supply: 24.5 cc. 9.75 per cent solution or 2.39 grams per hour = 0.94 gram per kgm. per hour.

These observations explain also the failure of Wierzuchowski and Gadomska (15a) to obtain constant responses in amytalized dogs, some of

which shivered violently during the glucose administration. They also suggest a possible source of error in experiments on conscious animals (15b) inasmuch as shivering is not an uncommon feature of continuous glucose administration under other conditions.

By way of control it has been observed repeatedly that the oxygen consumption and hence the approximate total rate of metabolism is practically constant in uniformly amytalized animals receiving glucose at constant rates (expt. 59, chart 8). Deuel, Chambers and Milhorat have published similar findings in amytalized dogs receiving no glucose, and have observed comparable increases of metabolism coincident with the onset of shivering (16).

It seems clear, therefore, that given a constant intravenous supply of glucose at a rate high enough to cause glycosuria, after a preliminary period required for equilibrium the glycosuria is fairly constant with uniformly maintained conditions, indicating a constant rate of utilization of the sugar injected. It also seems clear that the glycosuria is a more reliable index of the utilization than the blood-sugar concentration and that changes in the rate of utilization may be reflected quantitatively as variations in the rate of excretion. Further proof of this is contained in the data to be reported subsequently.

SUMMARY

When cats under the uniform influence of amytal anesthesia are supplied continuously with glucose by vein at constant rates, the chief characteristics of their response are as follows:

1. Abnormal glycosuria occurs on the average when the rate of supply exceeds 0.35 gram per kgm. per hour, increases with advancing rates of supply and tends to approach but not exceed 50 per cent of the supply with administration rates above 1.5 gram per kgm. hour. The response of any individual animal may vary as much as 15 per cent either way from these averages.

2. With any individual injection rate the glycosuria is fairly constant, after a preliminary period of two or three hours, for as long as nineteen hours and probably longer.

3. The blood-sugar concentration bears no such definable relation to the supply or the excretion of sugar in various animals. In any individual animal under constant conditions (especially of water supply) it is very constant.

4. Increased muscular activity (shivering), with consequent increased consumption of sugar, is reflected ultimately as a distinct reduction in the quantity of sugar excreted.

It may be concluded, therefore, that the use of these experimental conditions constitutes a reliable method for estimating the total utilization of sugar in acute experiments on unconscious animals.

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THE USE OF CONSTANT GLUCOSE INJECTIONS FOR THE STUDY OF INDUCED VARIATIONS IN CARBOHYDRATE METABOLISM

II. The Effects of Insulin, Pancreatectomy and Nervous Manipulations of the Pancreas

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From the Laboratories of Physiology in the Harvard Medical School

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It was reported in the preceding communication (1) that cats under the uniform influence of amytal anesthesia respond in a characteristic manner to the continuous administration of glucose at a constant rate. With a constant set of conditions and with an injection rate high enough to cause abnormal glycosuria (above 0.35 gram per kgm. per hour on the average), after an adjustment period of two or three hours a fairly constant concentration of blood sugar and rate of glucose excretion may be predicted in any experiment. An application of this knowledge is obvious, i.e., the blood and urine findings during constant glucose administration may be used advantageously as a direct measure of the rate of glucose utilization, and influences suspected of being able to modify normal utilization may be investigated in acute experiments.

The use of the method for this purpose is justified further by the following observations in which known factors, namely, insulin and pancreas removal, are shown to produce typical responses. The methods employed were described in detail in the preceding communication.

Insulin. Wierzuchowski (2) and Jordan (3) have shown previously that the administration of insulin to normal dogs receiving glucose at constant rates reduces the glycosuria and blood-sugar concentration. This is also true in amytalized cats, as shown by charts 1 and 2. (The findings for the preliminary hour or two of the injection are omitted from all charts.) Experiment 63 (chart 1) demonstrates the time relations involved with a single dose given gradually by vein. An appreciable glycosuria vanished and then reappeared. This response was demonstrated repeatedly. As little as 1 unit intravenously in a single dose may lower the glycosuria to a measurable extent. In the other two experiments reproduced (charts 2 and 3) the insulin was given continuously with the glucose. The effect

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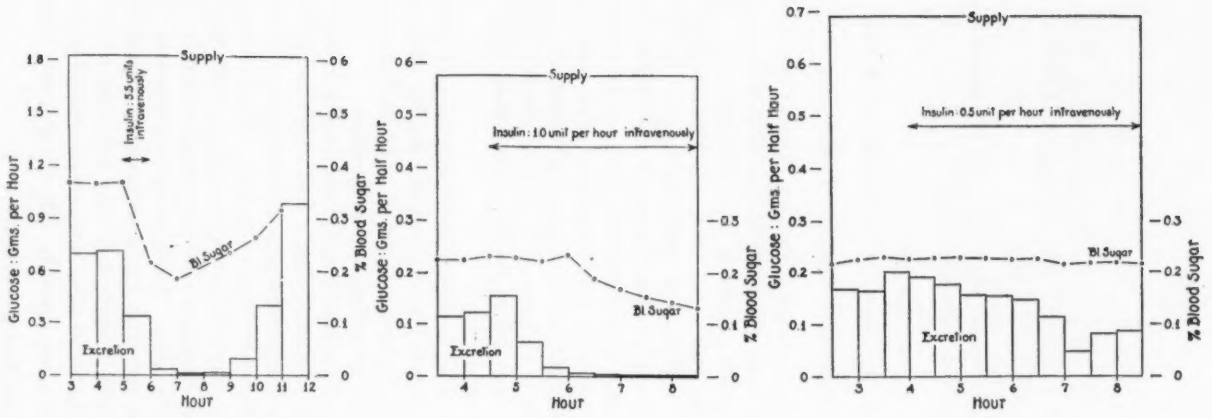

Chart 1 Chart 2 Chart 3

Effect of insulin on glucose excretion and blood-sugar concentration with constant supply of glucose. Chart 1. Experiment 63. Cat 276, weight 2.28 kgm. Glucose supply: 18.5 cc. 9.85 per cent solution or 1.82 grams per hour = 0.80 gram per kgm. per hour. Insulin mixed with glucose solution and hence given gradually during hour indicated.

Chart 2. Experiment 20. Cat 244, weight 2.38 kgm. Glucose supply: 12.2 cc. 9.35 per cent solution or 1.15 grams per hour = 0.48 gram per kgm. per hour.

Chart 3. Experiment 21. Cat 245, weight 3.10 kgm. Glucose supply: 14.4 cc. 9.57 per cent solution or 1.38 grams per hour = 0.45 gram per kgm. per hour.

is less striking because of the lower rates of administration but unmistakable nevertheless.

Of perhaps more than passing interest is the demonstration that insulin may reduce the glycosuria appreciably without affecting the concentration of sugar in the blood. The phenomenon is particularly striking in experiment 20 (chart 2) in which it is clear that the blood sugar was diminished only after the glycosuria had practically ceased. Since (assuming a constant blood volume) a constant blood-sugar concentration implies that the rate of entry into the blood is equal to the rate of removal from the blood, i.e., into the tissues and by way of the urine, the blood sugar might reasonably be expected not to diminish unless the increment of utilization result-

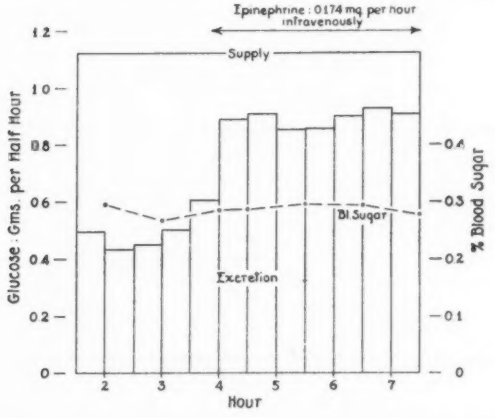

Chart 4. Effect of continuous epinephrine supply on glucose excretion and blood-sugar concentration. Experiment 64. Cat 277, weight 2.95 kgm. Glucose supply: 23.2 cc. 9.75 per cent solution or 2.26 grams per hour = 0.77 gram per kgm. per hour. Epinephrine supply (mixed with glucose solution): 0.174 mgm. per hour = 0.001 mgm. per kgm. per minute.

ing from the insulin should exceed the rate of glucose excretion. Under less carefully adjusted conditions such as those prevailing in experiment 63 (chart 1) and in ordinary diabetic management this feature might escape attention completely.

The reverse is illustrated by the experiment (chart 4) in which epinephrine, administered slowly at a constant rate along with the glucose solution, almost doubled the excretion of sugar without changing the concentration in the blood. If it is again assumed that the blood volume was essentially unchanged by epinephrine there can be no other explanation than that the extra sugar, whatever its source, was excreted as fast as it accumulated, inasmuch as the concentration in the blood did not rise.

However the phenomenon may be explained, the observations lend additional support to Woodyatt's view that glucose excretion does not depend solely upon the concentration of blood sugar (4).

Pancreatectomy. Woodyatt (5) has reported that the ratio between the supply and excretion of glucose approaches unity in depancreatized dogs. Wilder and Sansum (6) found the tolerance limit measured in this manner to be distinctly below normal in human diabetic individuals. Jordan (3) also observed high excretion rates in depancreatized dogs. The response indicated by these findings can be demonstrated clearly and promptly under the present conditions. In experiment 76 (chart 5) the pancreas was removed (with the attached duodenum) and in experiment

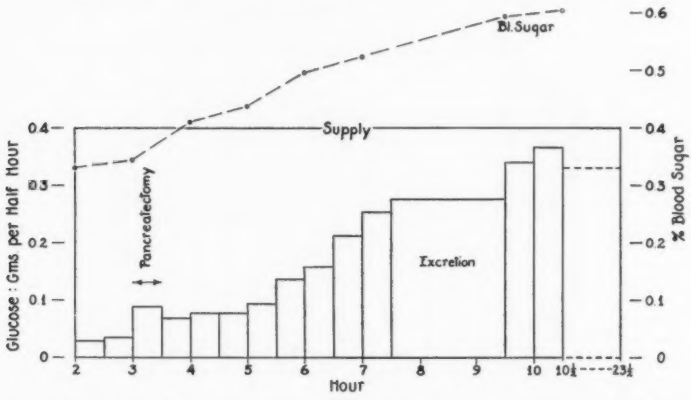

Chart 5. Removal of pancreas by excision results in total excretion of supply which causes little or no glycosuria normally. Experiment 76. Cat 289, weight 1.90 kgm. Pancreas excised at beginning of 3rd hour. Glucose supply: 8.0 cc. 10.0 per cent solution or 0.80 gram per hour = 0.42 gram per kgm. per hour.

85 (chart 6) its blood supply was tied off. During the subsequent few hours in both cases there was a progressively increasing glycosuria until in the seventh and fourth hours respectively almost all of the injected sugar appeared in the urine. The blood sugar was correspondingly but less strikingly increased. This response has been verified repeatedly. The consistency with which the excretion approximates the supply is notable. If the pancreatectomy is complete such a total excretion has invariably occurred within eight hours, usually more promptly, and in one case in which the pancreatectomy was only four-fifths complete because of the oversight of accessory pancreatic tissue in the splenic hilum, the excretion rose to only 65 per cent of the supply instead of the usual 90 per cent or more. In no case did the excretion exceed the supply.

Experiment 16 (chart 7) is presented by way of control for the operative

manipulations employed. Here an attempted pancreas ligation was incomplete, with the result that the blood and urine sugar maintained the constant relations always observed in uncomplicated experiments, in striking contrast to the findings after total pancreatectomy.

In view of the evidence presented in this and the preceding communication it seems reasonable to conclude that the method outlined constitutes a reliable measure for the rate of utilization of sugar, because first, in the absence of interference the response is constant and characteristic, and second, in the presence of influences known to be capable of augmenting or inhibiting the ability to utilize sugar, corresponding alterations are re-

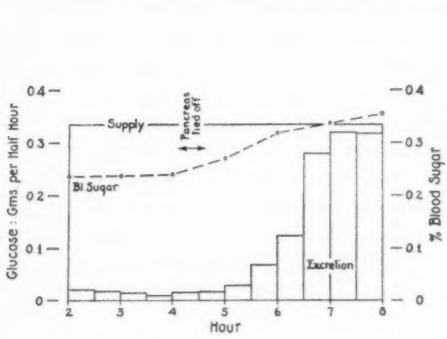

Chart 6

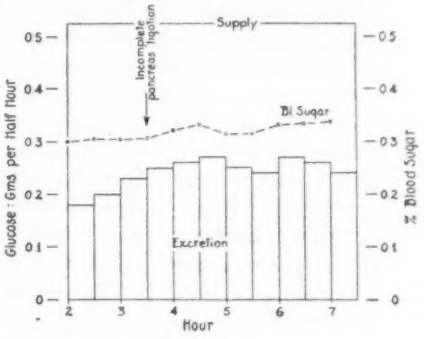

Chart 7

Chart 6. Removal of pancreas by ligation results in total excretion of supply which causes little or no glycosuria normally. Experiment 85. Cat 294, weight 1.91 kgm. Blood supply of pancreas tied off at beginning of 4th hour. Glucose supply: 10.0 cc. 6.70 per cent solution or 0.67 gram per hour = 0.35 gram per kgm. per hour.

Chart 7. Control for operative manipulations involved in pancreas removal. Experiment 16. Cat 236, weight 1.90 kgm. Glucose supply: 11.1 cc. 9.48 per cent solution or 1.05 grams per hour = 0.55 gram per kgm. per hour.

flected promptly and perhaps quantitatively by the method. Thus established, it appears possible by its use to obtain conclusive data concerning suspected or little known disturbances of carbohydrate metabolism.

As pointed out by Britton (7), the cells of the islands of Langerhans are provided with nervous connections according to the anatomical studies of Pensa, Gentes, de Castro and others (8). Various possibilities suggested by this fact have been tested by means of the present method. The balance of this paper is devoted to a discussion of the question of nervous control of insulin secretion and a description of experiments bearing on the problem.

The effect of bilateral vagus section. Studies by various investigators in-

dicate that vagus influences may be concerned with the regulation of insulin secretion and especially with an increased pancreatic response following the administration of glucose. Thus, Zunz and La Barre (9), using cross-transfusion preparations in which the insulin content of blood as it leaves the pancreas is measured (presumably) by the effect of a transfusion of a given quantity of it on the blood sugar of a normal or pancreatectomized recipient, have concluded that the administration of glucose stimulates insulin production by way of the vagus nerves. Häusler and Loewi (10) have come to similar conclusions in experiments based on the observation that the capacity of erythrocytes for taking up glucose *in vitro* appears to be proportional to the concentration of insulin in the mixture. Consistent hyperglycemia was observed by Papilian and Jianu (11) to follow section of both vagi, and Pollak's findings led him to believe that atropine inhibits the production of insulin after glucose administration (12).

On the other hand, Houssay, Lewis and Foglia (13), judging by the hyperglycemic responses of animals to single doses of glucose before and after bilateral vagotomy and inferior pancreatico-duodenal denervation, point out that the extrinsic nerve supply is not essential for the utilization of carbohydrates and that it probably plays a minor part in the regulation of the internal secretion of the gland. Still more discrepant are the findings of Clark (14) who observed indications of a temporary gain in sugar tolerance after section of the right vagus.

Other investigators have concluded that vagus stimulation may augment the secretion of insulin. De Corral (15), McCormick, Macleod and O'Brien (16), and Britton (7) observed hypoglycemia following prolonged electrical stimulation of the right vagus, and Britton's findings indicate that the result cannot be attributed to vasomotor influences. Ahlgren, working with a methylene blue decolorizing method (17), La Barre with his cross-transfusion method (18), and Hoshi, noting the effects of injecting extracts of blood treated by insulin separation methods (19), have confirmed this effect of vagus stimulation.

Clark (20) produced hypoglycemia by the administration of vagal stimulants such as guanidine and pilocarpine to rabbits under the influence of ergotoxine. In the presence of atropine or after section of the right vagus the response could not be elicited. Choline was found to have a similar action by Dresel and Zemmin (21), and Brugsch, Dresel and Lewy (22) observed hypoglycemia occasionally after injury of the anterior part of the vagus nucleus in the medulla.

It is difficult if not impossible to appraise these various results, owing to the variety of methods of biological assay involved, but the prominent fact remains that all investigators agree in their interpretations, with the possible exception of Lehmann (23) and Clark (14).

The method employed in the present study appeared to be adapted

favorably for a test of the function of the pancreatic nerve supply, inasmuch as this hypothetical vagus influence, if physiological, should be in operation with an excessive supply of glucose and its disappearance should be evident after vagus section.

Nine experiments of this type were performed. After isolation by gentle dissection of the vagus trunks as they enter the abdomen the response to the injection was allowed to come to equilibrium with the vagi intact. Both nerves were then cut and the effect on the glycosuria and blood sugar noted for at least four hours (in certain cases longer). In no experiment did section of the vagi alter the response of the animal to the glucose injection beyond the limits allowable with the method. Experiment 81 (chart 8) is typical of the results. After an immediate increase in glycosuria (interpreted as a result of transient sympathetic activity) the blood

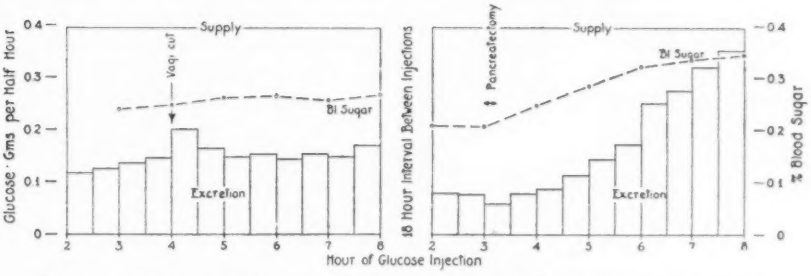

Chart 8. Bilateral section of vagus nerves does not affect glucose utilization but pancreatectomy later in same animal has typical effect. Experiment 81. Cat 292, weight 2.20 kgm. Laparotomy and isolation of vagi during first half-hour of injection. Vagi cut at point indicated by arrow. Pancreas removed at beginning of 3rd hour of second injection. Glucose supply: 8.8 cc. 9.01 per cent solution or 0.79 gram per hour = 0.36 gram per kgm. per hour (both injections).

and urine sugar remained at the levels of the control periods. The pancreas was removed from this animal twenty hours later with a characteristic response, showing that under these conditions the integrity of the vagus nerves is not a factor in the utilization of sugar, and presumably, therefore, in insulin secretion.

Whether or not the anesthesia altered conditions so that a normal response could not be elicited under these circumstances seems debatable. It is entirely possible that the diminished tolerance for sugar under amytal is due to the fact that the drug originally destroys all vagus activity. In this case cutting the vagi would have no further effect. Yet it should be pointed out that other signs of vagus depression, e.g., accelerated heart rate, are not present under amytal, and furthermore, the increased capacity for utilizing sugar occurring in the course of the adjustment to the in-

creased sugar supply may be demonstrated as easily in amytalized as normal animals. According to the prevailing view this latter adjustment is accomplished through the vagi and could not occur if they had been inactivated by the anesthetic. Finally, the observations of Zunz and La Barre were also made in acute experiments under anesthesia (chloralose) and they are the most convincing that have been reported. Thus, while it is difficult to reconcile the present findings with others on record, it may be concluded that under the present conditions, specifically in the anesthesia due to amytal, section of the vagi has no effect on the ability to utilize glucose. The effect of vagus stimulation was not tested.

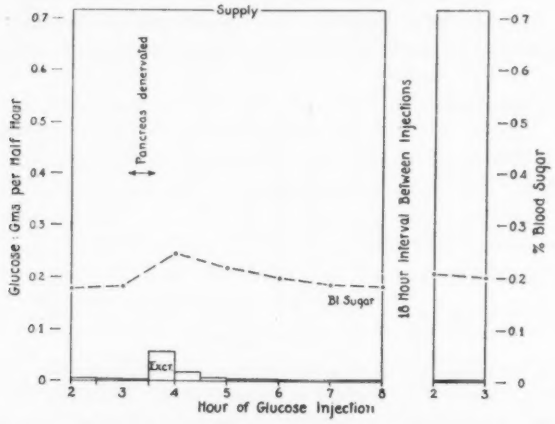

Chart 9. Denervation of pancreas has no permanent effect on glucose utilization. Experiment 91. Cat 299, weight 3.18 kgm. During 3rd hour of injection coeliac and superior mesenteric arteries denervated, mesentery between them cut, both vagi cut at diaphragm. Glucose supply: 14.4 cc. 9.90 per cent solution or 1.43 grams per hour = 0.45 gram per kgm. per hour (both injections).

Denervation of the pancreas. In three experiments the pancreas was completely denervated macroscopically by section of both vagi at the diaphragm, of the plexuses surrounding the superior mesenteric and coeliac arteries and of the mesentery between the arteries. This procedure results, as far as gross nervous connections are concerned, in complete isolation of the pancreas from the central nervous system. Experiment 91 (chart 9) shows the effect, which is negative except for the usual temporary disturbance probably due to mobilization of glycogen. The result is in accord with the fact that the pancreas may be transplanted without consequent diabetes (24).

Thus it may be concluded that induced nervous disturbances of a paraly-

tic nature do not alter the ability to utilize sugar and hence presumably the secretion of insulin, under the circumstances existing in the present investigations, i.e., in cats under the influence of amytal. The remaining possibility to be tested consists of noting the effect of stimulating the sympathetic fibers innervating the pancreas, with the hope of demonstrating an inhibitory action on insulin secretion.

The effect of prolonged splanchnic stimulation. Homans (25) and Britton (7) have recorded observations of this nature. Homans found evidences

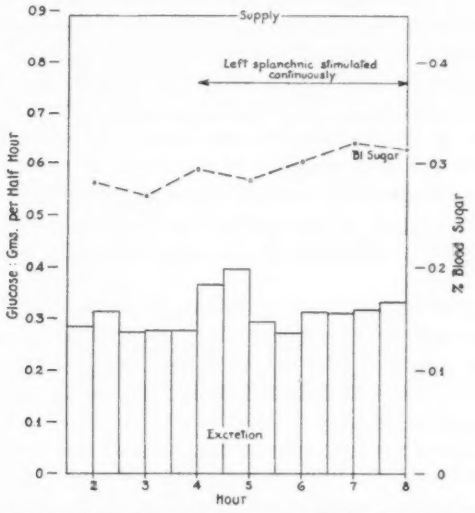

Chart 10. Negative effect of sympathetic stimulation in absence of adrenal and liver connections. Experiment 73. Cat 286, weight 2.23 kgm. Preparation 17 hours before start of injection consisted of excision left adrenal gland, section hepatic plexus and isolation left splanchnic nerve in abdomen. Continuous stimulation peripheral end left splanchnic from 4th hour on with weak induced 1½ volt shocks at intervals of about 1 second. Glucose supply: 17.8 cc. 10.0 per cent solution or 1.78 grams per hour = 0.80 gram per kgm. per hour.

of excessive epinephrine liberation after prolonged electrical stimulation of a major splanchnic nerve, but was unable to find histologically any sign of exhaustion of the pancreas islet cells. Britton stimulated the various sympathetic trunks containing pancreas fibers after eliminating the related mechanisms capable of causing glycogenolysis directly, namely, the adrenal gland in the circuit and the liver nerves, but was not able to produce hyperglycemia unless the hepatic plexus was undisturbed.

Four experiments similar to these of Britton's were performed. In order to eliminate the obscuring influence of the adrenals and the liver

nervous connections, the adrenal on the side of stimulation was removed and the plexus accompanying the hepatico-duodenal artery was severed fifteen or twenty hours before the experiment. Then after obtaining the customary control figures when equilibrium had occurred, the major splanchnic nerve was stimulated peripherally by means of repeated weak, short, induced electrical shocks over periods of three hours or more. The results were consistently negative. Experiment 73 (chart 10) is typical of the findings obtained in all cases. There was no measurable deviation of glycosuria or blood sugar from the control values except for the usual transient increase, in this instance probably due to unavoidable intra-abdominal manipulation when the electrodes were applied. It is true, of course, that section of the nerves along the hepatico-duodenal artery, in addition to disconnecting the liver from the source of stimulation (for which purpose it was performed) also severs those fibers entering the duodenum and pancreas along with the superior pancreatico-duodenal artery. Inasmuch as it was found to be technically difficult to disconnect the liver above the fork of this plexus, the superior pancreatico-duodenal nerve was stimulated separately in one experiment with similarly negative results.

While it is possible that none of these experiments satisfy the required conditions especially as to strength, frequency, duration, etc., of stimulation, it is clear that they afford no evidence that insulin secretion may be depressed by inhibitory nervous influences, but, insofar as they are applicable, indicate the contrary.

In recapitulation, then, it appears that various manipulations designed to alter the activity of the islets of Langerhans through their nerve supply, namely, splanchnic stimulation when the corresponding adrenal gland is absent, vagus section and complete pancreas denervation, appear to be entirely without influence on the functional capacity of the pancreas for internal secretion. Studies concerning the possibility that epinephrine may occupy an important position in this connection have proved to be more encouraging. The evidence to be reported in the last communication of this series shows that the continuous prolonged administration of epinephrine, in amounts which are well within the range of reflexly stimulated secretion, may suppress the ability to oxidize glucose even in the presence of an excessive supply.

SUMMARY

1. The administration of insulin diminishes, and of epinephrine increases, the excretion of sugar resulting from the constant injection of glucose by vein. With proper dosage these responses may occur with no corresponding changes in the blood-sugar concentration.

2. Complete removal of the pancreas causes virtually all of a constant

supply of glucose to be excreted in the urine. The effect appears gradually over a period of four to eight hours.

3. Under amytal anesthesia the ability to utilize glucose is not impaired by stimulation of the major splanchnic nerve after removal of the corresponding adrenal gland and section of the hepatic nerves, by denervation of the pancreas, or by bilateral vagus section.

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THE PROLONGATION OF PREGNANCY BY EXTRACTS OF CORPUS LUTEUM[1]

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That the ovarian factor responsible for the maintenance of pregnancy is the corpus luteum has been conclusively demonstrated by recent work. Ovariectomized animals have been shown to abort by Blair, Bell and Hick (2), Hammond (8), Drummond-Robinson and Asdell (5), Drips (4), Hartman (9), Nelson and Haterius (14), Herrick (10), and others. The results obtained by the extirpation of the ovaries are attributed to the absence of the corpus luteum by Hess (11), Wester (17), Parkes (15), and Nelson and Haterius (14).

The authors mentioned above employed purely operative procedures in one form or another. Little use has been made of the corpus luteum as a means of demonstrating its pregnancy maintaining function. While the present work was in progress Allen and Corner (1) reported that they had obtained an extract of the corpus luteum by the use of which they were able to maintain pregnancy in rabbits which had been deprived of their ovaries at the 18th hour of gestation. The authors were able to show that normal implantation and subsequent growth occurred in the bilaterally ovariectomized animals treated with the extract.

Teel (16) has reported that alkaline extracts of bovine anterior pituitary will bring about a lengthening of the gestation period to several days past the normal term for the rat. The fetuses were in all cases still-born. Evans and Simpson (7) have recently demonstrated that implants of fresh anterior pituitary glands will prolong gestation for several days past the normal period. The results are accounted for by the changes provoked in the host's ovaries, especially by the formation of fresh lutein tissue. The young when delivered were in some cases still-born and in others were viable. On the other hand Engle and Mermod (6) obtained abortion following such treatment. Long and Evans (13) have shown that histological changes occur in the corpus luteum of the rat shortly before parturition, and that these changes seem to indicate the cessation of function.

If the initiation of parturition is dependent upon the decline of the

[1] The expenses for this investigation were defrayed by a grant from the National Research Council, Committee on Research in Problems of Sex.

functional corpus luteum it should be possible to postpone parturition by supplying from an outside source the principle produced by the corpus luteum of pregnancy. Working on this theoretical basis the authors have attempted to show that the period of pregnancy may be prolonged and the time of parturition postponed by the subcutaneous injection of extracts of the corpus luteum.

TECHNICAL PROCEDURE. The animal utilized in this work was the albino rat. All of the females employed had borne at least one litter and in the majority of cases the duration of their gestation periods in previous pregnancies was known.

Pregnancies were calculated from the appearance of the vaginal plug and residual sperm. In all instances the actual act of copulation was observed. The day on which sperm was found in the vagina was called day 1, and the subsequent events were calculated thereform. Care was taken to note the appearance of the "placental sign" on the 12th to 14th day to make certain that successful implantation and growth of the embryos had taken place.

Injections of extract were begun in all instances on the 16th to 18th day of pregnancy. Cessation of injections varied in individual animals.

The length of the normal period of gestation is, of course, an important factor in work of this nature and can only be determined by the observation of previous pregnancies. In addition to the known records of individual rats, the average for the colony employed was taken as a control. This average was 21.5 days, with extremes of 20 days and 22 days. In no cases of which we have record did the term exceed 22 days. In the work reported here the time of delivery was known within two hours in all but two cases and those two were known within six hours.

A series of controls was run, treated in every way as the experimental animals except that the extract employed was one made of beef muscle, following the procedure used for the lutein extract.

The mother's behavior following parturition was observed in all cases to note whether any pronounced alteration of the maternal instincts occurred as a result of the treatment.

EXPERIMENTAL. Following the procedure outlined above fourteen pregnant rats divided into two series were treated with subcutaneous injections of the extracts. The preparation of the luteal extract employed in series A is as follows:

The freshly shelled corpora lutea (the luteal tissue was hand dissected within eight hours after removal of the sow ovaries) were thoroughly hashed and treated gradually with two and one-half volumes of methyl alcohol. After standing from 12 to 15 hours at room temperature with frequent agitation the mixture was strained through muslin and the glandular residue thoroughly expressed. The strained fluid was filtered and the fil-

trate evaporated before a fan at room temperature. The residue was suspended in normal saline solution so that 1 cc. represented 10 grams of fresh corpora lutea. An active extract also was obtained using methyl alcohol containing 1 per cent acetic acid, but the activity was less than that with methyl alcohol alone. Control extracts were prepared in the same manner from fresh muscle tissue. All extracts were stored at about 6°C.

Fresh extracts were prepared weekly, since it was discovered that they did not retain their potency for more than about eight days.

The results obtained in series A are summarized in table 1.

All of the mothers of this series except R-L_2 and L_1 exhibited normal maternal care for the young. The two exceptions refused to pay the slightest attention to their offspring, and it was only through the employment of a foster-mother that the litters were kept alive.

TABLE 1

Series A: Animals treated with methyl alcohol extracts

1 cc. equivalent to 10 grams corpora lutea

RAT NUMBER	INJECTION BEGUN (DAY OF GESTATION)	AMOUNT INJECTED PER DAY	INJECTION STOPPED (DAY OF GESTATION)	TOTAL AMOUNT INJECTED	PARTURITION (OVERTIME)	NUMBER OF YOUNG
		cc.		*cc.*	*hours*	
R_2L_1	17	0.5–1	22	4.5	36	10
R_1L-	17	0.5–1	22	4.5	70	5
R-L_2	17	0.5–1	21	3	30	8
R_1L_2	18	0.5–1	22	4.5	40	7
L_1	18	1	21	3	On time	11
R_4	17	1	22	5	48	8
R_3	17	0.5–1	22	4.5	48	9

A method for the further purification of the methyl alcoholic extract was devised using benzene and acetone. A typical experiment is included in order to give the details of preparation. Five hundred grams of fresh thoroughly hashed luteal tissue (sow) were treated as above with 1250 cc. methyl alcohol. After standing from 12 to 15 hours at room temperature with frequent agitation the mixture was strained through muslin and the glandular residue was thoroughly expressed. The strained fluid was filtered and concentrated in partial vacuo at 45°C. to a volume of about 75 cc. The gland residue from the first extraction was treated with 1000 cc. methyl alcohol and allowed to macerate at room temperature with frequent agitation for 24 hours. The solvent was removed in the same manner as in the first extraction. The vacuum distillation in this instance was continued until the residue was of a vaseline-like consistency.

The concentrate remaining after the first distillation was thoroughly extracted with 200 cc. benzene. After separation the aqueous phase was

discarded and the benzene solution was used to thoroughly extract the vaseline-like residue from the second distillation. To the filtered benzene solution were then added four volumes of acetone. The precipitate was filtered off, redissolved in 100 cc. benzene and again precipitated with four volumes of acetone. The precipitate was filtered off and discarded. The combined benzene-acetone solutions were freed of volatile solvents either in partial *vacuo* at 40°C. or in some instances they were removed before a fan. The semi-solid lipoidal residue was dissolved in a minimum of pure anhydrous ether (50 to 100 cc.) and about 25 cc. corn oil added. The ether was dissipated before a fan. Sufficient corn oil was then incorporated so that 1 cc. of extract represented 15 grams of fresh luteal tissue. The finished extract was stored in a refrigerator at about 6°C.

TABLE 2

Series B: Animals treated with benzene-acetone purified extract

1 cc. equivalent to 15 grams corpora lutea

RAT NUMBER	INJECTION BEGUN (DAY OF GESTATION)	AMOUNT INJECTED PER DAY	INJECTION STOPPED (DAY OF GESTATION)	TOTAL AMOUNT INJECTED	PARTURITION (OVERTIME)	NUMBER OF YOUNG
		cc.		*cc.*	*hours*	
R-L-	17	0.4	23	3	60	8
R_1L_2	17	0.6	23	3.5*	80	8
R_3L_3	16	0.4	22	2.8	48	2
R-L_1	16	0.4	22	2.8	65	10
R_2	17	0.4	22	2.5	48	8
L_3	17	0.45	24	3.5*	84	4
L_2	18	0.5	24	3†	150	4

* Still-born.

† Mother died with young *in utero*.

Control extracts were prepared in the same manner from fresh muscle tissue. Both luteal and control extracts were prepared fresh each week.

In some of the earlier work the semi-solid lipoidal residue was used as such but due to difficulties encountered upon injection it was decided to render the active fraction more mobile by dilution with an inert oil such as corn oil. The semi-solid lipoidal residue obtained from one kilo of luteal tissue weighs about 25 grams.

The results obtained in series B treated with this preparation are recorded in table 2.

All the rats of this series exhibited normal maternal responses toward their offspring. In all cases, except the two noted in table 2, the young were viable and all survived. The young borne 40 or more hours overtime have evidence of having attained a size somewhat greater than the average shown for offspring from a normal pregnancy.

Six rats were allowed to go through a normal pregnancy without treatment of any kind, the exact time of parturition being noted. In all instances birth took place within 3 hours of the colony average—21.5 days.

A series of six rats was treated with an extract of beef muscle prepared exactly as the benzene-acetone purification of the methyl alcohol extract had been. Amounts equivalent to the quantity of corpus luteum used were injected into these rats. Five of these animals delivered within a few hours of 21.5 days, while the sixth delivered at slightly more than 22 days. This single exception is explainable upon the slight normal difference in the time of gestation.

No pronounced systemic effect was noticed in the mothers treated with extracts until the pregnancy had gone 60 hours or more overtime, when a general state of disquietude and illness was discernible.

Discussion. These results present one more link in the chain of evidence for the pregnancy maintaining function of the corpus luteum. If, as Dixon and Marshall (3) believe, delivery is dependent upon the decline of the corpus luteum with subsequent follicular ripening, the continued presence of the active principle responsible for the inhibition of that process should postpone the event of parturition as described in the results of this work. Or if, as Knaus (12) has postulated, birth results from the greater susceptibility of the uterine muscles to pituitrin following the decline of the corpus luteum, a similar postponement should ensue if the responsible hormone is supplied from an external source. It is obvious that the described results would satisfy either theory inasmuch as the primary step in each is the disappearance of the lutein principle involved in pregnancy.

Of the two series it will be noted that the extract employed in B was the more efficient due in all probability to the higher percentage of the active principle contained therein. The average period of prolongation in series A was 45 hours, not including the one animal which delivered on time. This one exception (rat L_1) was no doubt due to the use of an extract that had stood for a week before being employed. As was noted during the study of the effect of this type of extract on the inhibition of oestrus there is a marked loss of potency when it is allowed to stand for a week or more. In series B the average prolongation was approximately 76 hours. Two of the litters were still-born (rats R_1L_2 and L_3). Both mothers had gone 80 or more hours overtime. From this and also from Teel's (19) results it is apparent that there is a limit to the time which fetuses may remain *in utero* and still remain viable. Rat L_2 failed to deliver at all and died after the pregnant period had been prolonged for 150 hours; she had shown evidences of growing progressively more ill after the third day. An autopsy revealed four fetuses in a state of decay, but still retaining evidence of having attained a size much larger than normal.

The normal functional activity of the mammary glands seemed in no way

impaired by the treatment, since the young thrived remarkably well. The failure of two of the mothers (rats R-L_2 and L_1) to care for their offspring is not surprising as occasionally an apparently normal mother will ignore her litter.

As may be noted by examining the tables there is no apparent relation between the number of young in the litter and the extent of the prolongation of the pregnancy.

While the prolongation of pregnancy for abnormal periods by the use of lutein extracts offers striking evidence for the rôle played by the corpus luteum in gestation, the authors realize that a truer criterion for the capacity for the maintenance of pregnancy upon the part of extracts of the corpus luteum would be the employment of them in animals bilaterally ovariectomized before the occurrence of implantation. Further work along this line is to be undertaken in the near future.

SUMMARY

1. Extracts of corpora lutea of pregnancy (sow) were successful, when administered subcutaneously, in prolonging the period of pregnancy in rats for periods ranging from 30 to 150 hours.

2. The young born 40 hours or more overtime gave evidence of a size larger than is characteristic of a pregnancy of normal duration.

3. If the period of prolongation did not exceed 70 hours the young were viable when born. In excess of this time stillbirths occurred.

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THE INFLUENCE OF SYMPATHETIC STIMULATION UPON INTRA-AURICULAR BLOCK IN THE MAMMALIAN HEART

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Observations upon the effects of sympathetic stimulation upon conduction in the turtle's heart led the author to compare the results obtained with those similar effects seen in the mammalian auricle after vagal stimulation which had been reported by Lewis, Drury and collaborators. The possibility was discussed that the effects in the mammalian heart might be explained as a result of the stimulation of sympathetic fibres coursing in the vagus nerve, although this explanation was difficult to reconcile with the evidence brought forward by the latter observers.

The old assumption of Gaskell (1886) that the action of the cardiac sympathetic was "perhaps to improve the conduction power of the different muscular tissues which together form the heart" has received support by Garrey's (1912–1925) interpretation of his observations upon the turtle's heart, by Gault (1917) and more definitely by Izquierdo (1928), both also working upon the turtle's heart.

At the moment sufficient data are not available of the effects of such stimulation upon all the different heart segments to determine whether this generalisation may apply also to the mammalian heart. The effects of such stimulation upon A-V conduction are compatible with such a view. All workers are in agreement that in both cold- and warm-blooded hearts sympathetic stimulation improves A-V conduction (Bayliss and Starling, 1892; Hunt, 1899; Hunt and Harrington, 1897; Dale and Mines, 1913; Routier; 1915a; Gault, 1917; Otto, 1927; etc.); that stimulation of its intracardiac endings by adrenalin (Routier, 1915b; Danielopolu and Danulesco, 1916, and others) has a similar effect.

When we consider the auricular muscle however the two observations, namely: the relief of intra-auricular block in the cold-blooded heart (Izquierdo, 1928) by sympathetic stimulation and the relief of a similar block in the mammalian heart by stimulation of the vagus nerve (Lewis, Drury and Bulger, 1921a, b; Lewis and Drury, 1923; Drury, 1925) make it clear that the generalisation does not hold for the mammalian heart if, in accord-

[1] Fellow of the Rockefeller Foundation.

ance with the evidence brought forward by Lewis, Drury and collaborators, the effect in the mammal is of pure vagal origin and not due to stimulation of sympathetic fibres in the vagus nerve, a point of view for which there is ample anatomical evidence (Billingsley and Ramson, 1918; Iwama, 1925). In consequence, the influence of sympathetic stimulation upon intra-auricular block has been inquired into, in the hope that the results might not only settle beyond dispute the reason for the relief of block seen in the mammal during stimulation of the vagus nerve but also throw some light upon the action of the sympathetic upon conduction through auricular muscle.

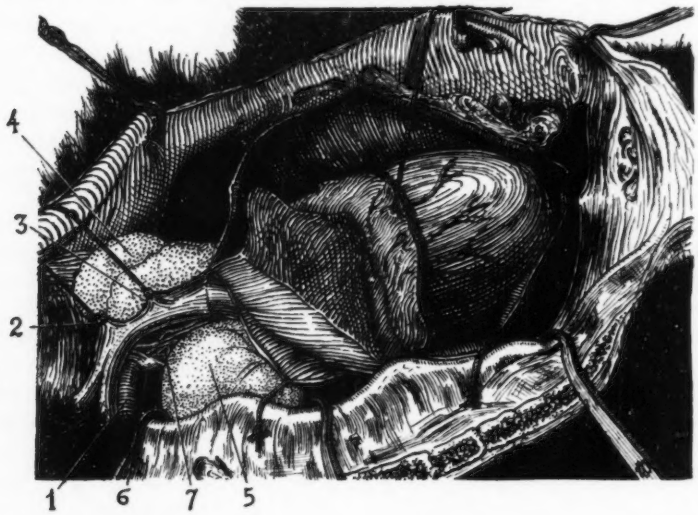

Fig. 1. Drawing to illustrate dissection of right cardiac sympathetic branches. *1*, right subclavian artery; *2*, costo-cervical vein; *3*, azygos vein; *4*, thymus gland; *5*, lung; *6*, right inferior cervical ganglion; *7*, cardiac branches.

METHOD. Dogs of about 10 kilos weight kept in a fasting condition overnight were anesthetised with morphia (1 ctgm. morphine sulphate per kilo weight) and chloralose, the latter being injected intravenously in doses of 10 cc. per kilo of a 10 per cent solution in saline. Under artificial respiration the chest was split and opened in the mid line, the thoracic walls pulled apart with hooks and the heart exposed, the pericardium being left unopened until the nerves had been dissected out.

In a few cases the first thoracic ganglia or their branches going to the ventral loops of the ansa Vieussenii were exposed by cutting the pleura covering them and were placed upon the stimulating electrodes. As the cardiac sympathetic fibres, however, originate from D_2 and D_3 and in small

part from D_1, D_4 and D_5 (Langley, 1892) and reach the heart not only through the first thoracic (stellate) ganglia but also through the inferior cervical ganglia (Hunt, 1899; Hering, 1923) they were usually dissected out nearer to the heart in the following manner (see fig. 1). The skin incision was carried upwards from the manubrium sternii for a few centimeters and the underlying muscle separated by blunt dissection. Both the azygos and costo-cervical veins were isolated and sectioned between ligatures and the superior vena cava lifted by fixing the ligatures attached to the central ends of the severed veins. The ansa Vieussenii which lies in the interspace between the brachio-cephalic vein and the subclavian artery, was located by carefully following the artery and the fine cardiac branches very carefully dissected as they originate from the ventral loop of the ansa, the ganglion, the recurrent and the vagus nerve itself. The number of such branches is extremely variable.[2] Owing to the admixture of vagal and sympathetic fibres in these branches they were stimulated in turn and the nerve selected which gave the best accelerator-augmentor effect.[3]

Usually the sympathetic effects produced by such stimulation are confused by others of vagal character due to stimulation of accompanying vagal fibres and these effects have been excluded completely by an intravenous injection of 1 to $1\frac{1}{2}$ cc. of 1 per cent solution of atropine sulphate. The nerve selected was placed upon shielded stimulating electrodes held by an adjustable clamp. It was found in agreement with other observers (Pawlow, 1887; Hunt, 1889; Langley, 1892; Muhm, 1901; Rothberger and Winterberg, 1910; etc.) that the nerves on the right side gave the larger effects, and they were consequently stimulated in the majority of the experiments. On occasions the left side was stimulated or the nerves on both sides were stimulated simultaneously. They were stimulated by strong faradic shocks at a rate of 10 to 25 per second, and usually for not longer than 4 to 5 seconds, as such stimulation gave definite and constant effects (see fig. 2). The duration of the experiment was usually about three hours. At the end of this time tests were made to ensure that the vagi were still inactive and the sympathetic nerves excitable.

After the sympathetic nerves had been prepared the pericardium was opened by a longitudinal incision, the free edges being sewed to the chest wall making a cradle in which the heart rested. The movements of the heart were limited by two threads, one passing from the fat in the auriculo-ventricular grove to the chest wall, and the other from the tip of the auricu-

[2] The six branches described by Dogiel (1911) were considered as 20 by Pawlow (1887); their number varies in my opinion according to the care in dissection.

[3] Such a branch belongs as a rule to the lower external group in which Pawlow (1887) found the main bulk of accelerator fibres, or to those marked 3 and 5 by Dogiel (1911).

lar appendix to a convenient point in the chest wall; in this manner the right auricular surface is well displayed and is free from excesive movement. To investigate conduction of the excitation wave through the auricular muscle, two sets of paired non-polarisable electrodes were placed in line with the stimulators, one set being placed on the tip of the appendix, the

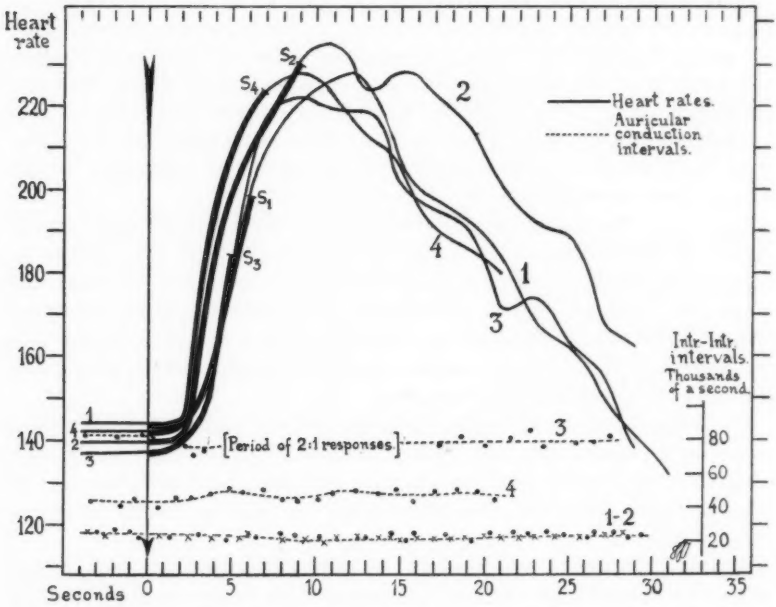

Fig. 2. Effects upon heart rate of four successive stimulations of right cardiac sympathetic nerves (dog WD, unatropinised). The commencement of stimulation is indicated by the arrow and the duration by the thick line in each curve. The heart rate is expressed in beats per minute calculated from the number of beats in each successive period of three seconds. The corresponding interintrinsic intervals (Intr-Intr) are plotted at the bottom of the figure. No pressure was exerted by the clamp during observations 1 to 2; definite pressure was exerted during observations 3 to 4.

other on the body of the auricle, in such a position that the excitation waves passed in a straight line beneath each pair in turn.

Each set of non-polarisable electrodes was connected to a galvanometer string and the arrival of the excitation wave at each simultaneously recorded. The stimulators[4] were fixed in the auricular muscle close to its junction with the inferior vena cava—or upon the inferior vena cava itself

[4] Consisting of two fish hooks cemented together with the least possible gap between them.

outside the pericardial attachment. To impair conduction between the recording electrodes a clamp 1 cm. wide with smooth jaws which could be approximated extremely slowly by turning a micrometric screw of 60 threads to the inch was carefully adjusted on the auricular appendix between them. The pressure was applied very gradually and great care was exercised only to test the effect of nerve stimulation when the degree of block produced was constant, and not to maintain severe pressure long enough to damage the muscle permanently. The observations were usually made with the auricle responding to shocks liberated at a rate just above the maximum rate reached by the normally beating heart during sympathetic stimulation. Parallel observations were made with the naturally beating heart as these gave an indication of the excitability of the sympathetic nerves throughout the experiment.

RESULTS. *General effects of sympathetic stimulation.* Preliminary experiments showed that in such a preparation sympathetic stimulation increased the heart rate very considerably, the maximum rate reached being about 220 to 240 per minute. The rise in rate is already noticeable during the stimulation, but the maximum is reached only 3 to 6 seconds after it is terminated (see figs. 2 and 3) and the effect lasts for about 30 to 40 seconds, for a stimulation of 4 seconds' duration.

In addition stimulation definitely increased the amplitude of the right auricular myogram obtained with a Cushny myograph both in the normally beating auricule and also in that responding to rhythmic shocks. The maximum increases of amplitude have been of about 35 to 60 per cent (see fig. 4).

Among the other reported effects of sympathetic stimulation, the decrease in the A-V intervals has been noted both in the normally beating or rhythmically driven heart.[5]

The well known changes in the P, R and T waves (Rothberger and Winterberg, 1910) have been constantly observed in the naturally beating heart.

Though I have not examined for these points specifically it appears from the inspection of the different records obtained that all the effects that have been associated with sympathetic stimulation are not seen equally developed in different hearts. In some the accelerator effects predominate, in others the augmentor, and so on.

At the moment I would call attention however to the close coincidence of their time relations: enhanced rate (see figs. 2 and 3); augmentation of the beats (see tracings in fig. 4) and changes in the electrical complexes reach their maximum within 3 to 6 seconds after the end of the stimulation

[5] In the heart driven rhythmically at a rate above that reached by the same heart during sympathetic stimulation, the P-R interval shows less definite shortening during the stimulation than in the naturally beating heart.

TABLE 1

Effects of sympathetic stimulation upon intraauricular block produced by pressure. Rhythmically driven hearts

DOG AND POSITION OF STIMULATING ELECTRODES	CONTROLLED AURICULAR RATE	PRESSURE EXERTED	SYMPATHETIC STIMULATED AND DURATION OF STIMULATION	INTRINSIC TO INTRINSIC INTERVALS		REMARKS
				Before	After	
				seconds	*seconds*	
WD, before atropine.	276	No pressure	Right; 4 seconds	0.041	0.038	Stimulation has no influence upon either 2:1, 3:1 or complete block
Body of auricle near inferior vena cava	246	Slight pressure		0.039	0.039	
	270	Pressure progressively increased		0.045	0.045	
	260			0.050	0.046	Stimulation increased heart rate from 100 to 200 per minute
	270			0.089	0.082	
	273			0.124	0.120	
	270			0.121	0.120	
WD, after atropine	236	Slight pressure	Right; 32 seconds	0.065	0.061	
					0.059	At the end of stimulation
					0.059	3 seconds after stimulation
					0.061	6 seconds after stimulation
					0.059	9 seconds after stimulation
					0.061	12 seconds after stimulation
						Stimulation increased the heart rate from 140 to 220 per minute
AAD, atropinised.	263	No pressure	Left; 4 seconds	0.043	0.045	Stimulation has no influence upon either 2:1, 3:1, or complete block
Body of auricle near inferior vena cava	260	Pressure progressively increased		0.055	0.055	
	264			0.057	0.056	
	264			0.115	0.106	Stimulation increased heart rate from 180 to 220 and produced definite T wave changes
BAD, atropinised.	214	No pressure	Right and left together; 4 seconds	0.057	0.063	Sympathetic stimulation increased the heart rate from 100 to 170 per minute and decreased P-R intervals
Body of auricle near inferior vena cava	220	Slight pressure		0.057	0.059	
	224	Pressure progressively increased		0.062	0.060	
	248			0.077	0.086	
	244			0.097	0.101	
	249			0.111	0.107	
	257			0.119	0.120	
	258			0.130	0.130	
	230			0.191	0.078*	
	231			0.156	0.103*	
	228			0.118	0.122	

TABLE 1—*Concluded*

DOG AND POSITION OF STIMULATING ELECTRODES	CONTROLLED AURICULAR RATE	PRESSURE EXERTED	SYMPATHETIC STIMULATED AND DURATION OF STIMULATION	INTRINSIC TO INTRINSIC INTERVALS		REMARKS
				Before	After	
				seconds	*seconds*	
CAD, atropinised. Outside the pericardium on inferior vena cava	210	No pressure	Right and left together; 4 seconds	0.025	0.027	Stimulation has no influence upon either 2:1, 3:1 or complete block
	200	Pressure progressively increased		0.034	0.031	
	230			0.043	0.046	
	214			0.049	0.052	
	220	Slightly reduced		0.040	0.044	Throughout the experiment sympathetic stimulation only gave slight acceleration; P and T changes were evident and the amplitude of the myogram was definitely increased (see fig. 4)

All the figures in this table correspond to 1:1 responses between the base and tip of the clamped auricular appendix.

All the measurements "after sympathetic stimulation" except those especially marked have been made when the sympathetic influence is maximal (see text).

used in these experiments[6] and in 30 to 40 seconds are practically over. This is important, because, as it was desired that the conduction intervals should be measured when the sympathetic influence was at its maximum, all measurements appearing in the tables as taken "during" sympathetic stimulation were systematically taken 5 seconds after the end of a 4-second stimulation.

The influence of sympathetic stimulation upon intra-auricular conduction. In table 1 are tabulated the results of stimulating the sympathetic upon intra-auricular block in four separate experiments. A close inspection of the figures shows that stimulation of either right or left sympathetic nerves or both right and left together has little or no effect upon the conduction of the excitation wave either through muscle which is normal, or through muscle in which conduction is impaired by pressure. In these experiments the effect of changing rate has been obviated by driving the auricle by rhythmic shocks throughout. To this conclusion that sympathetic stimulation has no effect upon conduction there is one major exception. In dog BAD at one stage of the experiment an improvement in conduction

[6] If the stimulation is more prolonged then they are seen to begin and reach their maximum during the stimulation (see fig. 2).

was registered (observations with*). No explanation of the improvement can be offered but it is difficult to associate this single exception with sympathetic stimulation as the maximal improvement was found to occur during or immediately following the stimulation, a period when as I have shown the sympathetic action is not, as judged by accelerator, augmentor of other effects, at its maximum.

TABLE 2

Effects of sympathetic stimulation upon stimulus-intrinsic intervals. Rhythmically driven hearts

DOG	POSITION OF STIMULATING ELECTRODES	CONTROLLED AURICULAR RATE	SYMPATHETIC STIMULATED AND DURATION OF STIMULATION	STIMULUS TO INTRINSIC INTERVALS		REMARKS
				Before	After*	
				seconds	*seconds*	
TD, atropinised	Body of auricle	276	Right; 4 seconds	0.044	0.045	
					0.047	1 minute later
	Body of auricle	312	Right; 4 seconds	0.042	0.037	
	Body of auricle	384	Right; 4 seconds	0.039	0.036	
					0.036	1 minute later
	Body of auricle	390	Right; 4 seconds	0.039	0.035	
	Body of auricle	424	Right; 4 seconds	0.041	0.041	
	Body of auricle	254	Right; 4 seconds	0.044	0.039	Acceleration from 196 to 210 per minute, upon stimulation
	Body of auricle	260	Right; 4 seconds	0.044	0.039	
AAD, atropinised	Body of auricle	264	Right; 4 seconds	0.021	0.022	Acceleration from 180 to 220 beats per minute; usual T changes in lead II, upon stimulation
	Body of auricle	258	Right; 4 seconds	0.021	0.021	
	Body of auricle	264	Right; 4 seconds	0.022	0.023	
	Body of auricle	264	Right; 4 seconds	0.023	0.023	

* Unless otherwise stated, the measurements in this column were taken 5 seconds after the end of a 4-seconds' sympathetic stimulation.

In table 2 the stimulus to intrinsic intervals before and during sympathetic stimulation in auricles beating at very different rates is tabulated. These intervals represent the time elapsing between the moment of stimulation and the arrival of the excitation wave at the recording contact and include not only the time taken for the wave to travel but also the latency of response. It is clear from this table that sympathetic stimulation has no influence upon such intervals. From table 1 it is evident that the stimula-

tion has no effect upon the rate at which the wave travels, and it can be concluded from these observations that it has no influence upon the latency of response at the rates of beating tested.

TABLE 3

Effects of sympathetic stimulation upon intra-auricular block produced by pressure. Naturally beating hearts

DOG	PRESSURE EXERTED	RIGHT SYMPATHETIC STIMULATED	BEFORE STIMULATION-		AFTER STIMULATION		
			Natural heart rate per minute	Intrinsic to intrinsic	Measurements made	Natural heart rate per minute	Intrinsic to intrinsic
		seconds		*seconds*			*seconds*
WD, unatropinised	No pressure	6	144	0 025	Immediately after stimulation	192	0 023
					3 seconds after stimulation	225	0 020
					6 seconds after stimulation	225	0 021
					9 seconds after stimulation	206	0 022
					12 seconds after stimulation	195	0 024
					15 seconds after stimulation	184	0 023
					18 seconds after stimulation	169	0 021
					21 seconds after stimulation	155	0 024
	No pressure	9	140	0 025	Immediately at the end of stimulation	230	0 022
					3 seconds after stimulation	235	0 024
					6 seconds after stimulation	228	0 024
					9 seconds after stimulation	219	0 023
					12 seconds after stimulation	201	0 025
					15 seconds after stimulation	189	0 024
					18 seconds after stimulation	168	0 023
	Definite pressure applied	5	137	0 081	Immediately after stimulation	184	2:1 response
					3 seconds after stimulation	222	2:1 response
					6 seconds after stimulation	219	2:1 response
					9 seconds after stimulation	217	2:1 response
					12 seconds after stimulation	195	2:1 response
					15 seconds after stimulation	183	2:1 response
					18 seconds after stimulation	170	0 081
					21 seconds after stimulation	158	0 079
					24 seconds after stimulation	138	0 081
					27 seconds after stimulation	140	0 084
	Pressure slightly reduced	7	142	0 044	Immediately after stimulation	224	0 044
					3 seconds after stimulation	227	0 049
					6 seconds after stimulation	214	0 046
					9 seconds after stimulation	196	0 049
					12 seconds after stimulation	186	0 045
ZD, atropinised	Definite pressure applied	4	135	0 109	During maximal influence	195	0 113

In all the experiments described the rate of auricular beating has been maintained constant throughout by means of rhythmic shocks applied to the auricular muscle. Such shocks are liable to stimulate locally the

sympathetic fibres running through the muscle, and consequently to produce an area of muscle constantly under sympathetic stimulation.[7] The limits of this area must vary from experiment to experiment, and it is possible that results may be vitiated in extreme cases by this area extending to the region of block. It is unreasonable to consider that such would occur in four successive experiments, especially as the stimulating electrodes were fixed as far away as possible either on the base of the auricle close to the inferior vena cava or on the inferior vena cava itself outside the pericardial attachment. Further evidence that these effects of this

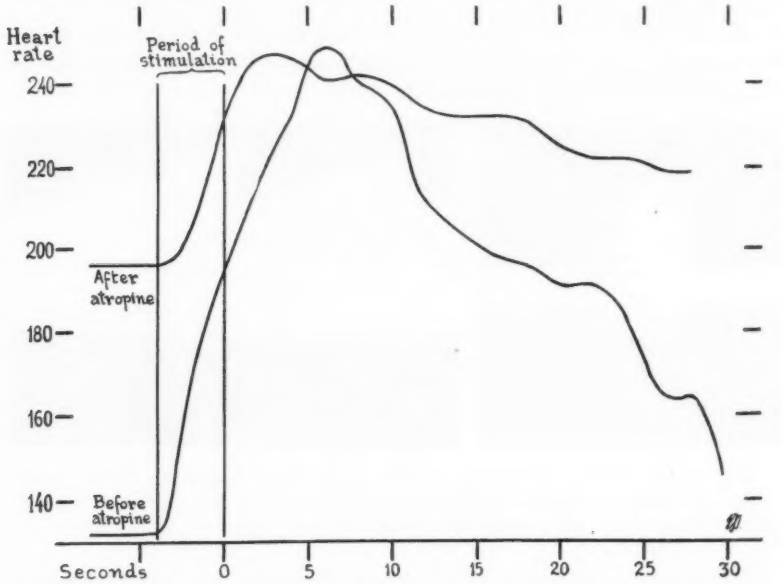

Fig. 3. Effect upon heart rate of strong faradic stimulation of the right cardiac sympathetic nerves before and after atropine. The heart rate is calculated as in figure 2.

stimulation are localised is supplied by the fact that the myogram of the rhythmically driven auricle is always increased in amplitude when the sympathetic nerves are stimulated.

In table 3 the results of sympathetic stimulation upon intra-auricular

[7] A few observations have been made upon the refractory period before and during sympathetic stimulation by the method described by Lewis, Drury and Bulger. As it is impossible at the moment to decide how far the effect of local sympathetic stimulation affects the results in such observations, it has been decided to omit the measurements.

conduction in the naturally beating heart are tabulated. From these figures it can be seen that in the unclamped auricle the conduction intervals at the higher rates are the same as at the lower rates of beating. If however sufficient pressure is applied to impair conduction, then with the heart beating at high rates, the block is increased and the tip of the appendix responds to every other bear of the auricle. This is to be ascribed to the higher rate of beating (Gaskell, 1886; Erlanger and Hirschfelder, 1905; Lewis and Oppenheimer, 1911) shortening the period of rest between the successive excitation waves and not to a further impairment of conduction in the compressed muscle.

All the observations which have been made show that sympathetic stimulation has little or no influence upon conduction through normal or compressed auricular muscle in the atropinised dog.[8]

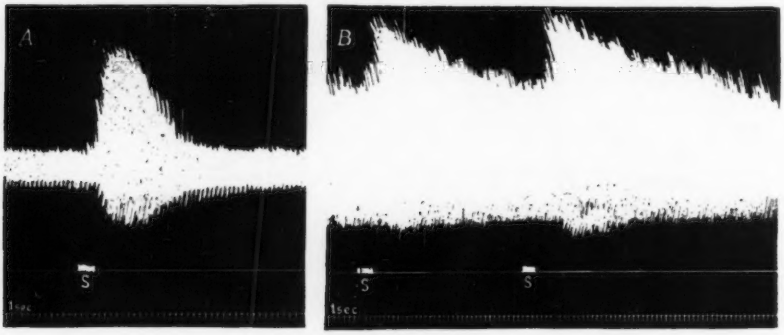

Fig. 4. Dog CAD, atropinised. Right auricular myograms to illustrate the augmentor effect of stimulation of the right cardiac sympathetic nerves A, naturally beating heart. B, rhythmically driven heart.

The action of atropine upon the sympathetic nerves. The great majority of the observations reported have been made upon dogs in which the vagi have been paralysed by atropine and large doses have been used so that no doubt should exist as to the complete paralysis of these nerves. It has been suggested (Backmann and Lundberg, 1922) that atropine paralyses not only the vagus nerves but also the sympathetic nerves. I have tested this by comparing the various effects of sympathetic stimulation before and after atropinisation.

It has been previously stated that when the sympathetic nerves are

[8] In dog WD sympathetic stimulation repeatedly gave pure accelerator effects and (see fig. 2) it was thought unnecessary to inject atropine. Nevertheless the stimulation had no influence upon conduction either through normal or damaged muscle.

stimulated for a period of four seconds the heart rate quickly reaches a maximum a few seconds after the end of stimulation and within a minute has returned to its previous level. After atropinisation a similar reaction is seen. In figure 3 is charted a representative example. The heart rates before and after stimulating the sympathetic nerve in a normal animal and for comparison the rates in the same animal after an injection of 1 cc. of 1 per cent solution of atropine sulphate are plotted. Before atropinisation the maximum rate reached after a strong faradic stimulation is about 240 to 250 per minute and the same rate is obtained after atropinisation while the duration of the reaction is slightly more prolonged. Observations have been made also upon the influence of atropine upon the augmentor effects. The effect has been investigated throughout several experiments and it is found that the amplitude of the auricular myogram is always increased by sympathetic stimulation in the fully atropinised ani-

TABLE 4

Influence of atropine upon amplitude of auricular myogram. Rhythmically driven heart at 250 per minute

AMOUNT INJECTED	TOTAL AMOUNT INJECTED	PERCENTAGE INCREASE AFTER SYMPATHETIC STIMULATION
mgm.	*mgm.*	
1.0	1.0	43
1.0	2.0	51
1.0	3.0	60
2.0	5.0	58
2.5	7.5	35
2.5	10.0	54
5.0	15.0	56
5.0	20.0	60

mal. This effect is obtained in hearts beating naturally or in those in which auricle is driven throughout by rhythmic shocks from a constant point in the auricular muscle (fig. 4A and B). In dog FAD the amplitude of the vagal auricular myogram of the auricle responding to rhythmic shocks at a rate of 250 per minute, has been investigated before and after several successive doses of atropine, and the percentage change is tabulated in table 4.

Before the injection of atropine the amplitude was decreased owing to predominance of the vagal effect but after the injection of 10 to 15 mgm. the amplitude was still increased by approximately 50 to 60 per cent which is very comparable with the increases after the initial small doses. Rothberger and Winterberg (1910) have described the changes in size and form of the P, R and T waves consequent upon sympathetic stimulation and I have carefully examined the records of lead II and have been unable to

detect that atropinisation has any influence upon these changes. The decrease in A-V intervals is equally well seen after atropinisation.

There is every reason therefore to consider that the doses of atropine used have exerted no paralysing effects upon the sympathetic nerves and the conclusion can be safely drawn that the observations reported in the earlier part of this paper are of effects in auricular muscle under the influence of sympathetic stimulation.

Influence of stimulating the vagus nerve at its point of emergence from the skull. The observations already reported left little doubt that stimulation of the sympathetic nerve did not influence intra-auricular block in the atropinised heart. In addition no proof could be obtained of the suggested paralysing effect of atropine upon the sympathetic nerve. A further observation was made however, namely, to test the influence of stimulating the vagus nerve as it emerges from the skull upon normal and impaired conduction.

The right vagus nerve was dissected out very carefully as it emerges from the foramen jugulare. A fine silk ligature was tied as high as possible and the nerve severed above it. The heart was prepared in the usual manner and the clamp fixed in position on the right auricular appendix.

Stimulation of the nerve as close as possible to the ligature was followed immediately by relief of block, whether this block was one of prolonged conduction intervals, 2:1 response of the tip of the auricle or complete block. In the latter circumstance a full 1:1 response of the tip was immediately brought about. No change in normal conduction was observed. The effects were, in fact, identical with those obtained by Lewis, Drury and Bulger (1921a, b), Lewis and Drury (1923) and Drury (1925) by stimulating the vagus nerve high in the neck or by the injection of acetylcholine.

It cannot be argued that pure vagal stimulation is obtained by stimulating the nerve as it emerges from the skull as a few sympathetic fibres join it at this point. This experiment is in itself strong evidence, however, that the relief cannot be due to sympathetic stimulation for the results obtained are identical whether many fibres, as in the case of stimulating the vagus nerve high in the neck, or very few, as it emerges from the skull are stimulated. It is only reasonable to consider that the relief is due to vagal stimulation, especially as it has been shown in this paper that stimulation of the sympathetic nerve itself has no influence.

Discussion. It has been conclusively shown that sympathetic stimulation has no definite influence upon the rate at which the excitation waves are conducted through auricular muscle, whether they are travelling at the usual rates through normal muscle or whether they are travelling slowly through muscle subjected to pressure. Moreover if the pressure is increased until every excitation waves fails to pass through, such stimulation leaves the block unchanged. Owing to the admixture of vagal fibres in the sympathetic it is necessary to fully atropinise the animal to

exclude the vagal effects but no evidence could be obtained that such a procedure in any way paralyses the activity of the sympathetic fibres. Lewis, Drury and collaborators have shown that by stimulating the vagus nerve high in the neck the excitation waves travelling slowly through compressed muscle are speeded up. Moreover when the pressure is sufficient to prevent all the excitation waves from passing through, such stimulation enables every excitation wave to pass, a complete block being converted into a 1:1 response. They brought forward evidence that this effect was due to the stimulation of vagal fibres. The observations here reported make their explanation the more certain, for it can no longer be argued their effects were due to stimulation of sympathetic fibres coursing in the vagus nerve, for whatever anatomical evidence may exist as to the presence of such fibres, stimulation of the sympathetic nerve itself is without effect upon intra-auricular block. The above authors, moreover, have shown that atropine completely abolishes the reaction; this drug is known to paralyse the vagus nerve and, as has been shown in this paper, in the doses employed has no influence upon the effects of sympathetic stimulation. If additional evidence is required, it is supplied by the experiment in an unatropinised animal, reported here, in which the vagus nerve was stimulated at its exit from the skull.

There remains no doubt therefore that no other explanation can be offered of the relief of intra-auricular block in the mammalian heart by stimulating the vagus nerve in the neck than that offered by the authors quoted, namely, that it is a vagal effect.

It would appear that the favourable action of sympathetic stimulation upon conduction in the A-V bundle and the tissues forming the basal segments of the cold-blooded heart is not duplicated in the mammalian heart. It is true that A-V conduction is improved[9] but conduction in the auricular muscle is unaffected by such stimulation. The improvement of conduction in the mammalian auricle is a function of the vagus nerve which acts in a contrary sense in the cold-blooded heart. At the moment it is evident that no observations made upon the effects of vagal or sympathetic stimulation on the cold-blooded heart can be transferred without investigation to the mammalian heart. It has been already stated in relation to vagal stimulation and I may now add as a result of these and previous observations (Izquierdo, 1928) on sympathetic stimulation "that a fundamental difference exists between reptilian and mammalian hearts" (Lewis, Drury and Bulger, 1921). It is of great importance that further precise work should be carried out in both types of heart so that data may be available not only to make a comparative study but also to judge how far improvement of conduction can be considered as a "characteristic of sympathetic stimulation."

[9] It is doubted that it improves ventricular conduction (Wiggers, 1927).

SUMMARY AND CONCLUSIONS

As intra-auricular block has been found to be relieved by sympathetic stimulation in the cold-blooded heart and by stimulation of the vagus nerve in the mammalian heart, the effects of stimulating the sympathetic nerves upon conduction through mammalian auricular muscle have been investigated.

The effects usually associated with right or left sympathetic stimulation have been found to be developed in different hearts in varying degree.

Faradic stimulation of the right, left or both sympathetic nerves has no definite effect upon the conduction of the excitation wave through normal or compressed muscle, whether the heart is beating naturally or responding to rhythmic shocks.

Atropine must be employed to exclude the effect due to stimulation of vagal fibres accompanying the sympathetic nerves; this drug in the doses employed has no influence upon the effects of sympathetic stimulation.

The relief of block already reported as a result of stimulation of the vagus nerve high in the neck, has been duplicated by stimulating the same nerve as it emerges from the skull.

The relief of intra-auricular block seen in mammalian hearts after stimulation of the vagus nerve must therefore be, as has already been suggested, a pure vagal effect.

I wish to express my warmest thanks to Dr. A. N. Drury for the generous help and advice he has given me throughout this work. I am also indebted to Professor Dean for permitting me to carry out this investigation in his Department.

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FILLING OF THE NORMAL HUMAN HEART IN RELATION TO THE CARDIO-PNEUMOGRAM AND ABDOMINAL PLETHYSMOGRAM

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If the venous blood returned to the chest at a *constant rate*, there would at the end of systole be less blood in the chest than there was at the beginning of systole. If we let Q = the *net* amount of blood which leaves the chest during systole, i.e., the difference between the amount of blood in the chest at the beginning and at the end of systole, then

$$Q = S - (V + P) \tag{1}$$

where S = the stroke volume of the left ventricle, 75 cc. or more.

V = The return during systole through the veins entering the chest, which, since systole is approximately one-third the cycle, would on the above assumption of constant inflow equal 25 cc. or more, and

P = the net systolic expansion of the intrathoracic arteries belonging to the systemic circulation.

Bramwell, Downing and Hill (1923) have shown that large human arteries (carotid) expand 6 per cent to 7 per cent when subjected to a sudden change in pressure from 75 mm. (diastolic) to 125 mm. (systolic). Dealing as we are with the net systolic increase in arterial volume, the difference between the pressure at the end of systole and that at the beginning of systole would be less than the pulse pressure and hence the volume change would be less than the above figure. A convenient estimate would be 10 cc. or less.

Substituting the above values in (1) we find Q = 40 cc. or more.

Now if the respiratory passages are open Q would be replaced by air moving into the chest during systole. An equivalent amount of air (40 cc. or more) would perforce move out again during diastole. These events would involve a respiratory volume of about 3 liters per minute and disregarding the dead space could be thought of as replacing respiratory movements, or even respiratory regulation, since cardiac and respiratory regulation are roughly parallel, *cf.* Landois' (1891, p. 100) remarks concerning hibernating animals.

Of course such a cardio-respiratory mechanism would involve serious difficulties in vocalization and would be quite inconvenient if the respiratory passages (e.g., glottis) should be closed; for then the cardiac volume changes above described would produce changes in the intrathoracic pressure of from 7 to 10 mm. Hg.

Ordinary experience indicates that no such intrathoracic volume and pressure changes relate themselves to the cardiac cycle in man although this fact seems to be lost sight of occasionally in the literature (John Hunter, 1835; Klewitz and Baumm, 1921; Wiedemann, 1919; Wiggers, 1923, p. 92).

A rather large literature on cardiac changes in intrathoracic pressure has arisen. The literature on the cardio-pneumogram, as it is called, is reviewed by Luciani (1911) and Klewitz (1918). It is the intention here to reopen the subject in the hope of arriving at a rough quantitative estimate of Q, the *net* amount of blood which leaves the chest during systole; which is equal of course to another net quantity, the amount of blood which enters the chest during diastole.

METHODS. The tracings of the intrathoracic pressure here described were made by optical registration. The manometers were fashioned by flattening one side of a brass tube, stretching a piece of condom rubber over the end, tying with thread and sealing with shellac. An optically adequate mirror was made by silvering the flat side of a thin weak lens such as was formerly used to cement on spectacles for bifocal glasses. The curved front surface served to focus an image of the illuminant upon the slit of an electrocardiographic camera and threw no front surface images. The illuminant was an ordinary 100 watt, clear glass, gas filled bulb with a C-shaped filament. This was clamped so that the image was foreshortened into a straight vertical line.

With the focusing lens on the capsule no difficulty was experienced in avoiding time parallax by setting the two capsules (pulse and intrathoracic pressure recorders) in the same horizontal plane. The manometer was calibrated by forcing air, 1 cc. at a time, into an 18 liter flask which was connected with the instrument. The deflections were recorded and the pressures calculated from the volume relationships.

The pulse was taken with a glycerine pad over the brachial artery and transmitted to a less sensitive (more rapid) capsule similar to the one already described. Records of intrathoracic pressure changes due to cardiac activity were taken by putting a tube, leading to the capsule, in the mouth, respiring to a passive expiratory standstill through the nose and closing the internal nares with the soft palate. This position could be maintained after practice for 10 or 15 seconds without involuntary respiratory movements distorting the record. Such movements could be recognized when the records of two successive cardiac cycles presented marked dissimilarities of outline.

A typical record is seen in figure 1 *a–c*. The notch *a* is simultaneous with the R wave and occurs with its subsequent rise during the period of isometric contraction. As the period of ejection begins there is a rapid fall in intrathoracic pressure. This lasts 0.04 to 0.05 second, whereas systole as measured on the brachial pulse curve or electrocardiogram lasts about six times as long. During the first sixth of systole, blood is leaving the chest. During the rest of systole, blood is entering the chest more rapidly than it is leaving since the point *b* which marks the end of systole on the cardiopneumogram is always higher than the lowest point of the early systolic fall. Further, during the whole of diastole very little blood (or none at all, see fig. 3) enters the chest. Considering the intrathoracic cardiovascular system as a whole, all of diastole is diastasis!

If the chest were a rigid box it would be possible from the pressure change *c–d* to calculate how much blood leaves the chest (minus the inflow) in early

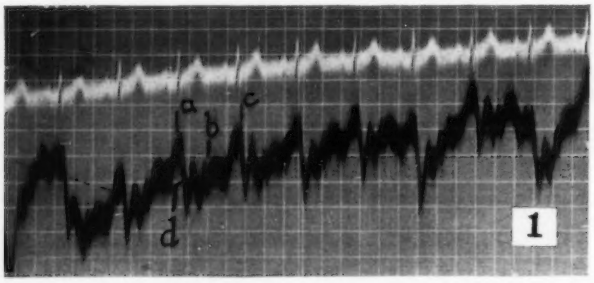

Fig. 1. Simultaneous tracing of intrathoracic pressure (lower) and electrocardiogram (upper). Letters indicate approximately: *a*, period of isometric contraction; *b*, end of systole; *c*, beginning of active systole; *d*, beginning of rapid venous return to auricles. The broad line is due to use of flat mirror (cover glass).

systole and from *d–b* how much reënters (minus the outflow). This would be a simple application of the gas laws. The pressure effect of blood flowing in and reducing the air volume in the chest is modified, however, by the elastic give of the chest walls and diaphragm.

Wiedemann (1919) and later Klewitz and Baumm in 1921 have attempted to evaluate the "stroke volume" of the heart by mathematical deductions from the pressure changes. Since an accurate record of the pressure changes shows that the pressure goes down only during the first sixth of systole, it is plain that blood is entering the chest faster than it leaves during the last five-sixths of systole and that one could not assume anything as to the whole stroke volume from the first sixth of the stroke even if we could assume that the early pressure changes then were due exclusively to myocardia.

It is possible, very roughly, to get an idea of how much more blood is in the chest at *c* that at *d* by following Wiedemann's procedure. This procedure gives the maximum value possible.

The rounded pressure curves at the left of figure 2 were recorded with a bottle containing 18 liters of air between the mouth and the capsule. At *X* the bottle was closed off from the lung-capsule system. The sharper,

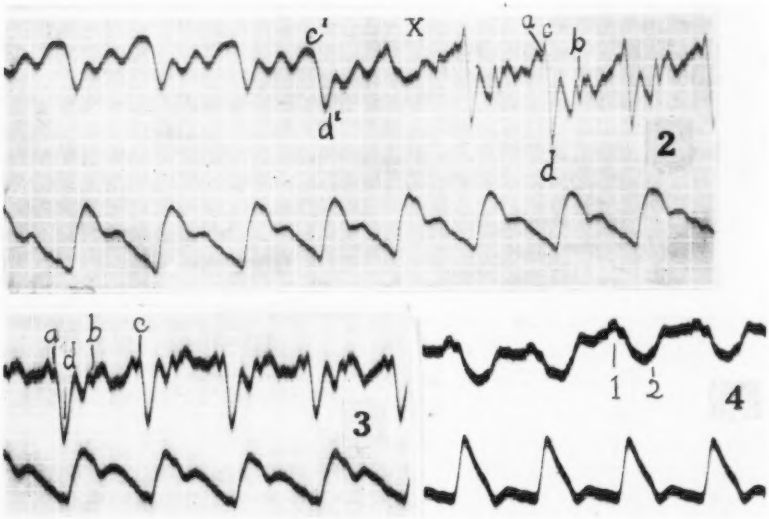

Fig. 2. Simultaneous tracing of intrathoracic pressure (upper) and brachial pulse (lower). Letters as in figure 1. There seems to be no return of blood to the chest during diastole. Mirror as described in text.

Fig. 3. Simultaneous tracing of intrathoracic pressure (upper) and brachial pulse (lower). The rounded curves at the left (upper tracing) were taken with an 18 l. air bottle between the mouth and the recording capsule. At *X* the bottle was removed from the system.

Fig. 4. Simultaneous tracing of the radial pulse (lower) and abdominal plethysmogram (upper). *1*, first heart sound vibrations; *2*, second heart sound vibrations. The abdomen becomes smaller during systole.

larger pressure changes at the right were made immediately succeeding cardiac cycles but without the bottle. The normal pressure change of 0.24 mm. Hg (*cd*) is reduced to 0.14 mm. Hg (*c' d'*) by the elastic give of the air in the bottle.

Now if the elasticity of the larger volume of air were the only factor to be considered Wiedemann's equation would apply and we would be able to show by substituting the experimental values and solving the equation

that there is 7.5 cc. more blood in the chest at c than at d and that during diastole the intrathoracic vascular system fills up only to the extent of 3 or 4 cc. (fig. 2) or not at all (fig. 3).

The rigid application of Wiedemann's equation is not justifiable because of inertia and damping effect of the air in the bottle clearly recognizable in the rounded peaks of the curves published here and by Klewitz and Baumm (1921). Another factor that may enter is an upward mechanically produced movement of the diaphragm during systole (Mackenzie, 1913) which would vitiate the assumptions of Wiedemann's equation. The inertia of the heavy chest walls is a third factor which is not included in Wiedemann's assumptions. This author's equation implies that the volume changes of the cardiac cycle produce the same pressure changes in this case as they would in 23 liters of confined air and thus neglects the restricting effect of the chest walls whose inertia would make them fairly resistant to quick pressure changes.

If the chest walls had the elastic and inertia properties of confined air the volume change responsible for c–d would be 7.5 cc. Now if the chest walls were quite rigid, the pressure change c–d could be accounted for by a volume change of 0.75 cc., assuming a mid-capacity of 2.3 liters. The true volume change which causes this pressure change is probably closer to the lower figure.

This is borne out by the following observation. When one studies a saliva film across the lips or a soap film in a tube held in the mouth, one can clearly recognize oscillations of cardiac origin, provided of course that the glottis is open, the internal nares closed, and respiratory movements held in abeyance. These oscillations represent volume changes with pressure changes reduced to a minimum, but they (the oscillations) are only 1 or 2 cc. in amplitude. Since these volume oscillations are obviously of cardiac origin and are of small magnitude, the inference is that the total quantity of blood in the chest does not vary much during the cardiac cycle.

Returning to the relationships expressed by

$$Q = S - (V + P)$$

we must conclude that since Q is insignificant and P a small quantity, V and S must be about equal to each other. In other words, the systolic inflow into the chest nearly equals the systolic outflow from the chest, minus the pulsable expansion of the intrathoracic arteries. Thus the venous return to the chest must show a pulsatility which is nearly synchronous with the pulsatile outflow; the difference between the two being shown in the relatively small volume changes which are responsible for the cardio-pneumogram.

The rate of blood flow into the chest is determined by two factors: 1,

the pressure from behind, and 2, the resistance from in front. The pressure from behind is the "effective venous pressure" (Henderson and Barringer, 1913) i.e., the difference between venous and intrathoracic pressure. This is of course relatively constant throughout a cardiac cycle. The resistance from in front must be sufficient to obstruct flow into the chest. Auricles and intrathoracic veins must therefore be distended in early diastole and the ventricles filled by forces arising in the elastic recoil of the (veins?) auricles, and later in their contraction. These forces would not only push blood into the relaxing ventricle but also pull the ventricular walls around the entering blood.

During systole there is a sudden reduction in the resistance from in front of the venous flow, because nearly all of the systolic movement tends to enlarge the venous reservoirs in the thorax. The following considerations will make this clear:

1. The forces which produce the apex beat hold the front of the heart firmly against the chest wall during systole.

2. Atmospheric pressure holds the lower surface of the heart in contact with the diaphragm. The systolic pull upon the diaphragm (Mackenzie, 1913) can produce but little movement upward because of the inertia of the heavy abdominal organs.

3. There can be but little net movement medially of the walls of the heart which are in contact with the lungs because such movements would be directly impressed upon the cardiopneumogram. This, however, indicates a systolic increase in lung volume that is negligible.

4. Having excluded other possibilities, we are left to assume that the major systolic movements of the ventricular walls are a forward movement of the back wall and a downward movement of the upper wall. These walls are in contact with the great venous reservoirs, auricle and venae cavae, and the ventricular movements described would enlarge these reservoirs in proportion to the extent of the movements.

This would allow, during systole, a rush of blood from the extra thoracic venous reservoirs into the chest impelled by the "effective venous pressure," and permitted by the increased capacity of the intrathoracic venous reservoirs. Jugular blood moves into the chest during systole as is seen from the depression after the *c* wave of the venous pulse curve which shows a systolic emptying into the chest of the blood distending the jugulars. That the abdominal venous reservoirs empty (into the chest) during systole can be demonstrated by abdominal plethysmography.

To make the abdominal plethysmograph a sheet of lead was cut, hammered and bent until it conformed to the contour of the abdomen, reaching from the pubis and groin to the costal margins, and from one mid-axillary line to the other. Around the rim of this lead "abdomen" was cemented a large soft rubber tube. To the tube in turn was cemented

a sheet of rubber dam making an air tight space between the lead wall and the abdominal wall to receive the abdominal pulsations. The pressure changes made by changes in abdominal volume were recorded optically.

The upper tracing in figure 4 is the abdominal plethysmogram and the lower curve is a simultaneous pulse tracing (distorted for convenience in recording, by a small leak). The figures 1 and 2 point out the vibrations of the first and second heart sounds. In spite of arterial expansion the size of the abdomen diminishes every time the heart beats. The most reasonable way of accounting for this seems to be by assuming that the abdominal venous reservoirs empty during systole in accordance with the above outlined mechanism, into the thoracic venous reservoirs.

If the argument so far presents no glaring fallacies it must be concluded that during the whole of diastole the intrathoracic veins and right auricle are distended with a sufficient head of pressure to prevent further movement of blood into the chest until the next systole. Even when the right ventricle is filling there can be no appreciable movement of vein blood toward the heart since this would involve flow of blood into the chest and a marked rise in intrathoracic pressure.

All the blood that goes to the heart goes there during systole. During diastole the venous reservoirs are wholly distended and rigidly full.

Diastolic filling of the ventricle is essentially a transfer of blood from auricle to ventricle (*cf.* Harvey). The heart as a whole, assuming that right and left hearts behave similarly, maintains a relatively constant volume.

From this standpoint the problem of whether the heart is adequately or inadequately filled may shift to a new basis, since ventricular systole brings blood to the auricles in an amount quantitatively similar to that sent out through the aorta. Shifts in the rate of ventricular relaxation due to nervous and nutritional changes may play a major part in the problem.

Thus if the ventricle should relax slowly and hence incompletely, transfer of blood from auricle to ventricle would be hindered, the auricles would increase in size and the ventricles decrease. On the other hand if the ventricles relax rapidly and hence more completely, they would increase in size at the expense of the auricles.

It may be that since the x-ray silhouette does not differentiate between auricle and ventricle, we have here a key to the difficulties encountered when we find that a normal resting dog's heart throws the same shadow when beating at a rate of 60 as it does at 110 (Meek, 1924), and at the same time puts out at the rapid rate much less blood per beat (Marshall, 1926). It may be that the slow heart with the large stroke volume has large ventricles and small auricles and that the rapid ventricle with less time to relax, takes a smaller proportion of the auricular blood at each beat. More

blood would be in the auricles of the rapid heart but this fact could not be proven by x-rays.

A similar problem, in its theoretical background, is encountered when we assume (Henderson and Haggard, 1925, and most other authorities) that the stroke volume of man is increased during exercise, but that the diastolic heart shadow remains about the same (Meek and Eyster, 1923).

It would not be in accord with classical physiology to assume that a ventricle may increase its rate and at the same time either increase or decrease the force and completeness of its contraction without any change in diastolic size.

This conflict would be resolved by assuming that the diastolic heart shadow is no index to the size of the living ventricle beating in contact with the auricle.

It should be pointed out that for anatomical reasons all that has been said of the human heart cannot be completely transferred even to the normal dog. If the chest is open, as has been necessary in plethysmographic studies, the forces and anatomical relationships which produce systolic filling of the auricle would be destroyed. Since these forces and relationships are important in the normal action of the heart, they should not be destroyed by experimental procedures unless due account is taken of their absence.

SUMMARY

Study of the changes produced in the intrathoracic pressure of man by the cardiac movements indicates that during ventricular systole an amount of blood nearly equal to the systolic discharge enters the auricles and that little or no blood flows to the heart during diastole.

Some theoretical implications of these findings are discussed.

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